Inflammation and immunomodulation in uveal melanoma

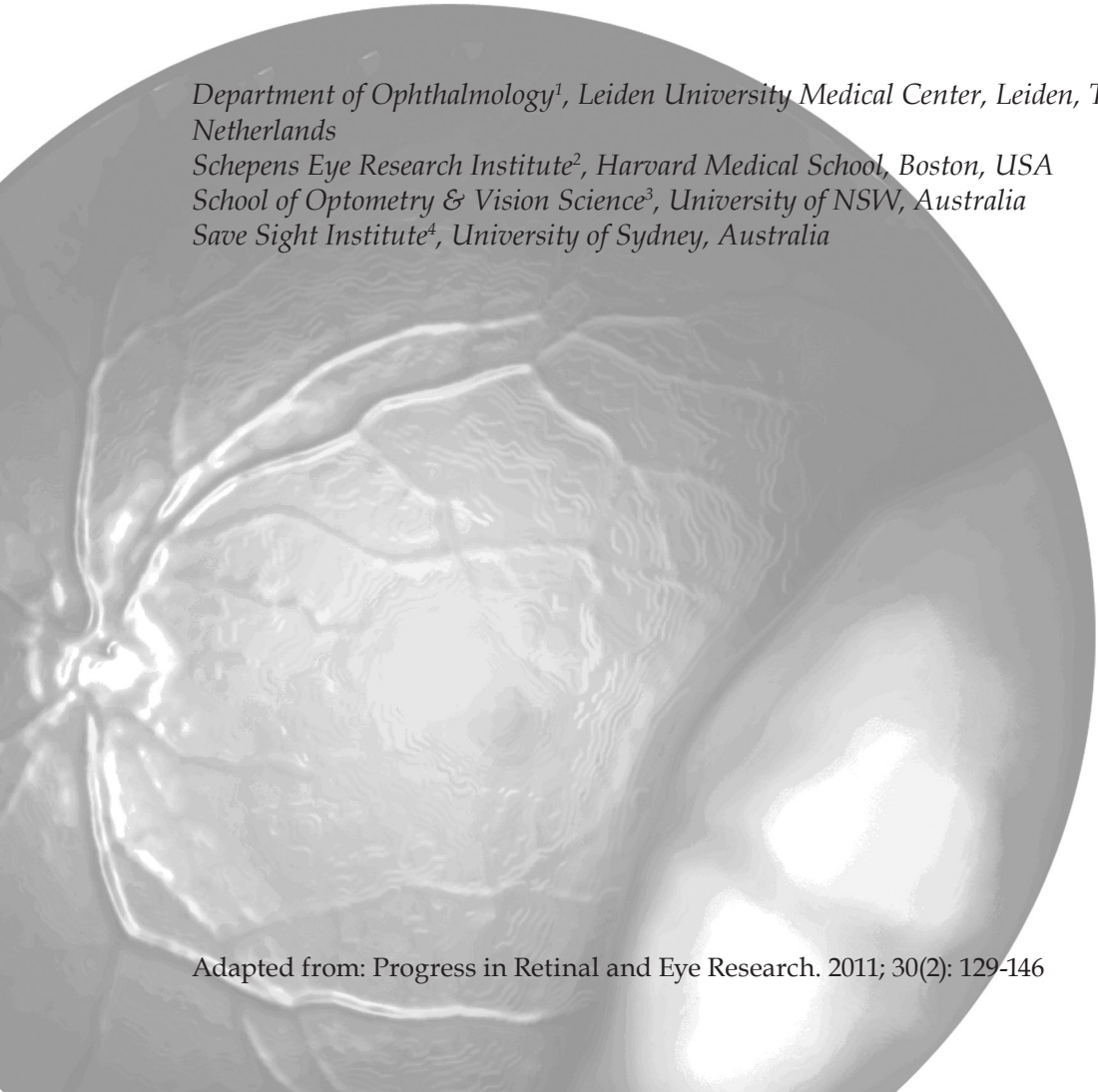
Ly, L.V.

Chapter 2

Macrophages in uveal melanoma and in experimental ocular tumor models: Friends or foes?

Martine J. Jager[1,2], Long V. Ly[1], Mariam el Filali[1], and Michele C. Madigan[3,4]

Department of Ophthalmology[1], Leiden University Medical Center, Leiden, The Netherlands
Schepens Eye Research Institute[2], Harvard Medical School, Boston, USA
School of Optometry & Vision Science[3], University of NSW, Australia
Save Sight Institute[4], University of Sydney, Australia



Adapted from: Progress in Retinal and Eye Research. 2011; 30(2): 129-146

Abstract

Macrophages belong to the innate immune system and as such constitute one of the first barriers against infection. They play an important role in wound healing, in inflammation and in angiogenesis, but are also essential in the first stage of a "danger response". After scavenging debris, they can digest cellular proteins into smaller pieces, and protein-derived peptides can subsequently be presented to the immune system. Depending on the activation state of the macrophage, this antigen presentation may trigger a full-blown active immune response, or may suppress a potential immune reaction. Macrophages constitute a heterogeneous cell population described by many names, with varying phenotypic characteristics, depending on their tissue location and state of activation. They play important roles in different ocular tissues, including the cornea and the choroid, and have been found to be involved in anti-tumor immune responses in mouse ocular tumor models. One would thus expect macrophages to belong to the "good guys" that help to protect our body against dangers such as cancer. In human uveal melanoma however, a high density of macrophages is associated with a poor prognosis for the patient. Macrophages play a role in promoting angiogenesis, and thus may stimulate tumor growth; in addition, macrophages have also been found to suppress anti-melanoma immune responses. These functions may shift during aging. Taken together, these new observations extend our understanding of the diverse functions of macrophages and show us their different faces, making them either "friends or foes" in human uveal melanoma. A better understanding of these multifaceted cells will help in developing new treatments to prevent the growth of metastases in uveal melanoma patients.

Article Outline

1. Introduction

Macrophages originate in the bone marrow, where their earliest identifiable progenitor is the myelomonocytic stem cell. In the bone marrow, monoblasts proliferate and differentiate along the macrophage lineage up to the monocyte stage. Monocytes move to the blood and from there into many different tissues, where they develop into mature macrophages[1]. Metchnikoff first described macrophages over 100 years ago, and delineated a characteristic function: removal of debris by engulfing it[2]. Since then, many subtypes of bone marrow-derived precursor cells, peripheral blood monocytes, and tissue macrophages have been described, that collectively constitute the mononuclear phagocyte system[3]. What do these cells actually do? In the first place, as already mentioned, macrophages can function as scavengers, removing debris after trauma or infection. However, they also play important roles in tissue remodelling and in innate immunity[4]. During infections, they can become activated, and being present in all tissues, thus constitute the first line of defence. Using a multitude of cell-surface receptors and cytokines, macrophages can act as antigen-presenting cells (APCs), stimulating reactions of the adaptive immune system.

Within the eye, macrophages are of great importance as seen for example in the defence against corneal infections, including those caused by bacteria[5], Herpes Simplex Virus Type 1[6], Acanthamoeba[7], and fungi[8]. Furthermore, they are essential in the early phase of corneal allograft rejection [9]: removal of macrophages prior to corneal transplantation reduced graft rejection to almost zero, most likely due to the fact that when macrophages were absent, antigen presentation no longer occurred.

Macrophages may not only stimulate immune responses, but as myeloid-derived suppressor cells (MDSCs), they can also inhibit the development of effective immune responses or provide help to induce tolerance. This phenomenon has been observed in auto-immune diseases such as experimental auto-immune uveoretinitis and in cancer [10,11]. When macrophages are present within tumors, they are often referred to as tumor-associated macrophages (TAMs). In a specific type of intraocular tumor, i.e. uveal melanoma, a higher density of macrophages is associated with a worse patient prognosis quod vitam[12]. This brings us to a fourth function of macrophages: macrophages may be important in cancer due to their regulation of angiogenesis (see below). Tumors need blood vessels for sustenance and growth, and macrophages may not only be involved in stimulating the development of new vessels, but can have an angiogenesis-inhibiting function as well. An example can be found in ocular tumors: when macrophages were removed in an intraocular tumor model in old mice, tumor growth was almost completely prevented[13].

As new characteristics of specific macrophage subpopulations continue to be discovered, their paradoxical functions become increasingly evident. The current paradigm proposes a polarisation of macrophages related to their overall effect and phenotype, with distinct M1 and M2 macrophages being described [14-16]. The different functions of M1 and M2 macrophages and their possible role(s) in uveal melanoma will be discussed and we will describe the

presence of macrophages in uveal melanoma in relation to histologic findings and survival. We will also discuss the role of macrophages in angiogenesis and in ocular immunology, analysing different animal models. As uveal melanoma is a deadly tumor, obtaining a better understanding of the growth of uveal melanoma and metastasis and the potential contribution of macrophages may help to develop more appropriate therapies for uveal melanoma.

2. Different types of macrophages

Macrophages may directly lyse hostile cells or pathogenic organisms by releasing toxic substances. This specific "killing" function is characteristic of CD14+ myeloid progenitor cells, and is stimulated by pro-inflammatory signals such as Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), and bacterial products such as lipopolysaccharide (LPS), and Interferon (IFN)-gamma which is produced by other immune cells. Macrophages that have such immunostimulatory functions, anti-bactericidal activity and release of inflammatory cytokines, are now classified as M1-type macrophages [15]. Such macrophages function as Antigen-Presenting Cells (APCs), and thus help T and B cell to develop immune responses against a multitude of infectious agents and against transplanted tissues.
Mantovani et al. [15] introduced the concept of another subset of macrophages – M2 macrophages – that is mainly involved in enhancing angiogenesis, tissue repair, extracellular matrix (ECM) remodelling, and immune suppression within the tumor. Tissue homeostasis needs to be restored following damage induced by an immunological reaction, and M2 macrophages play an essential role in this process [17]. These cells are of special interest to ophthalmology because of their pro-angiogenic capability, and the significant impact of neovascularisation on vision. Macrophage-Colony Stimulating Factor (M-CSF), IL-4, and IL-13 are essential cytokines that stimulate myeloid progenitor cells to differentiate into M2 cells.
M2 macrophages can produce matrix metalloproteinases (MMPs, such as MMP-9) and secrete growth factors including vascular endothelial growth factor (VEGF), emphasising their key role in tissue remodelling and angiogenesis [18]. Besides restoring local physiological tissue functions, M2-type macrophages are also immunosuppressive. One can imagine that during tissue repair, the development of inflammation is counterproductive. During local wound healing, M2 macrophages counteract, at the same time, any stimulatory signal that might lead to a strong immune response. This immunosuppressive function can be created by triggers such as corticosteroids and the suppressive cytokine IL-10, which stimulates M2 cells to further produce IL-10, leading to a suppressive feedback loop.
The identification of two subtypes of macrophages is especially relevant for tumor biology, since macrophages are frequently found in solid tumors. M1-type macrophages are able to kill cells, and have the potential to suppress tumor growth, while M2-type macrophages with their pro-angiogenic capability and ability to suppress immune responses are expected to favour tumor

progression. Some studies claim that TAMs are mainly of the M2-macrophage subtype, and that the degree of infiltration with these cells can be determined by counting the number of CD163+ cells. The presence of CD68-positive (a marker that characterises cells of the monocyte/macrophage lineage) TAMs in malignancies is generally considered an indicator of poor prognosis [19] and these cells are considered to be tumor-promoting. The most important ocular tumor in adults, the uveal melanoma, contains TAMs. Uveal melanoma carrying moderate or high concentrations of TAMs carried a bad prognosis [12,20]. The possible reasons behind this finding are the subject of this review.

3. Immune cells in uveal melanoma

Uveal melanoma is the most common primary intraocular tumor in adults, with an estimated annual incidence of 6–10 cases per million per year in Caucasian populations [21,22]. It is a tumor that can develop in the iris, the ciliary body or the choroid, and especially occurs in adults and the elderly. Up to 50% of patients with uveal melanoma may die from metastatic disease[23,24], and while an increased incidence in the occurrence of metastases is observed 2–3 years after recognition of the primary tumor, metastases may still develop after 10–15 years, and even after 35 years (Fig. 1). The most frequent site of metastases is the liver, and aside from sporadic successful local chemoperfusion and occasional liver surgery for a few small metastases, no effective therapy for metastatic disease exists to date [25-27]. Once metastases are identified, the median time to death is 10–18 months [26,28]. In order to identify mechanisms that play a role in prognosis and tumor growth, we can learn much from studying primary tumors and compare those that are malignant with those that are not. Many histological parameters have been identified that are related to prognosis (Fig. 2), and these include the presence of infiltrating lymphocytes and macrophages [29,30]. Tumors may contain variable levels of infiltrating lymphocytes, which in uveal melanoma consists mainly of T cells; several early studies identified the presence of lymphocytes in uveal melanomas as being a bad prognostic sign [31-34]. The presence of a subtype of T cells, the FOXP3-positive T cells, was recently described to occur in one out of four uveal melanoma, and was a marker of worse survival [35]. De Waard-Siebinga et al. [36] analysed the presence of different types of infiltrating leukocytes in uveal melanoma, and found CD3+, CD4+, and CD8+ lymphocytes, as well as CD11b+ and CD15+ cells, considered to be monocytes/macrophages and granulocytes, respectively. CD15+ cells were rare, but CD11b+ cells were present in ~90% of tumors studied. B cells were seen in only one case. A positive correlation was observed between the presence of CD3+ cells, CD11b+ cells, and the level of HLA Class I expression (Fig. 3). Interestingly, local treatment with thermotherapy was noticed to further stimulate the influx of macrophages [37]. It is assumed that this influx of scavenger cells helps to remove tumor cells after thermotherapy/brachytherapy. Intra-tumoral macrophages often carry pigment and are called melanophages.

3.1. Macrophages in uveal melanoma

As mentioned above, different macrophages can be identified. Markers that have been used to identify macrophages in humans include CD68 and CD163 [39]. The hemoglobin-haptoglobin scavenger receptor protein (CD163/HbSR) is a monocyte/macrophage restricted transmembrane glycoprotein of the scavenger receptor-rich family. It reacts with mature monocytes and macrophages and with neoplastic cells of monocyte/histiocytic origin, and also stains tissue macrophages [40,41].
Mäkitie et al. [12] used CD68 as a marker to study the relationship between the density of TAMs and patient survival in uveal melanoma in 149 cases of choroidal or ciliary body melanoma enucleated between 1972 and 1981. The tumors were divided into three groups: those with a few (17% of cases), a moderate number (51% of cases) or many macrophages (32% of cases). Higher numbers of TAMs were associated with more patients dying from uveal melanoma metastases. Higher numbers of TAMs were also associated with female gender, larger basal tumor dimensions, epithelioid cell type, heavy pigmentation, and a high microvascular density (MVD), thus with bad tumor phenotypes [42]. An association between the presence of a high number of TAMs and a high MVD has also been observed in other malignancies such as cutaneous melanoma [43] and breast cancer [44], suggesting a causal relationship.

3.2. Other antigen-presenting cells in uveal melanoma

In order to obtain efficient T cell priming, a series of events needs to take place, including sufficient antigen presentation on professional APCs. Several studies have shown that many types of APCs are present in uveal melanoma, among which are macrophages and dendritic cells. Dendritic cells (DCs) can be further classified into subtypes by studying their morphology and surface antigen expression [45]. Markers identifying different APCs include FXIIIa, expressed on DC (independent of maturity), CD68 (expressed by tissue macrophages and DCs), HLA-DR (essential for antigen presentation, expressed on activated macrophages), CD40 (a molecule essential for interaction with T cells), and CD83 (marker for mature DCs). Obviously, there is an overlap in these characteristics between DCs and macrophages.
Cells carrying the markers FXIIIa, CD68, and HLA-DR are generally found in uveal melanoma, indicating that, indeed, DCs and macrophages are present [45]. These APCs were most often located at the border of the tumor adjacent to normal tissue, or close to the vasculature. However, CD40, considered to be essential for stimulating T cells, was not expressed on DCs, but occasionally on pigmented melanophages, while CD83, a marker for DCs that are able to prime T cells, was only seen on some cells in one out of ten cases studied [45]. Since the maturation and activation markers CD40 and CD83 are required for antigen presentation, lack of these markers may interfere with the development of a proper T cell-mediated anti-tumor immune response in the eye. However, as the APCs neither express TGFβ nor indolamine 2,3 dioxygenase (IDO), which are both necessary for the induction of tolerance, it may be that they

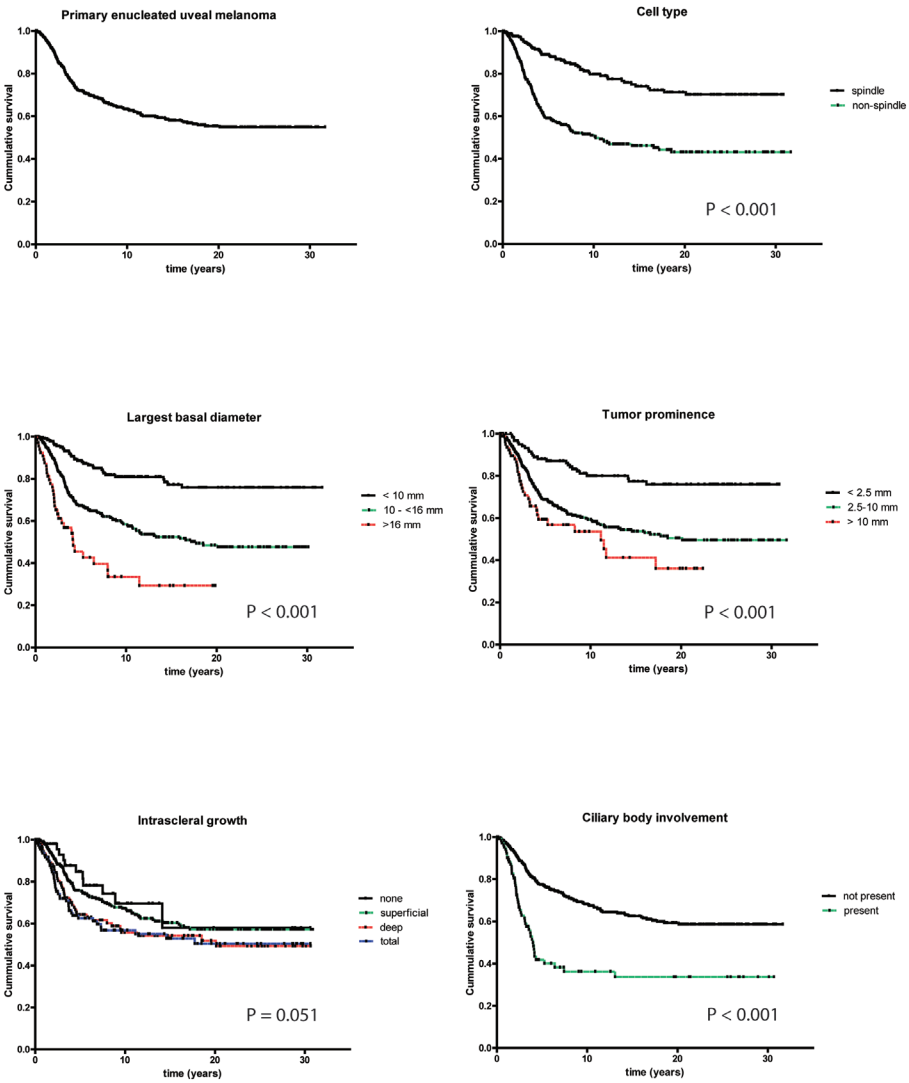


Fig. 1. Survival in relation to specific parameters of 643 cases of uveal melanoma that underwent primary enucleation at the LUMC between 1972 and 2007. Shown are the survival curves of all cases, and survival in relation to cell type, largest basal diameter, tumor prominence, intrascleral ingrowth and ciliary body involvement.

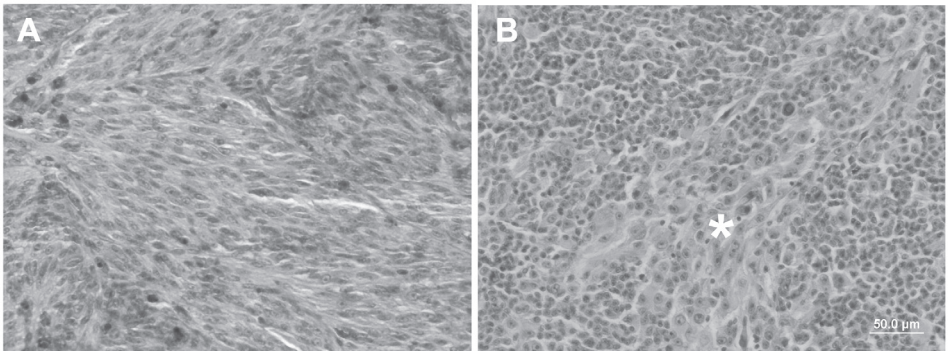


Fig. 2. Histological example of different cell types. Light micrographs showing **(A)** spindle cell and **(B)** mixed (spindle and epithelioid) cell morphology in uveal melanoma [38]. Note the epithelioid cells (*) in **(B)** (H&E).

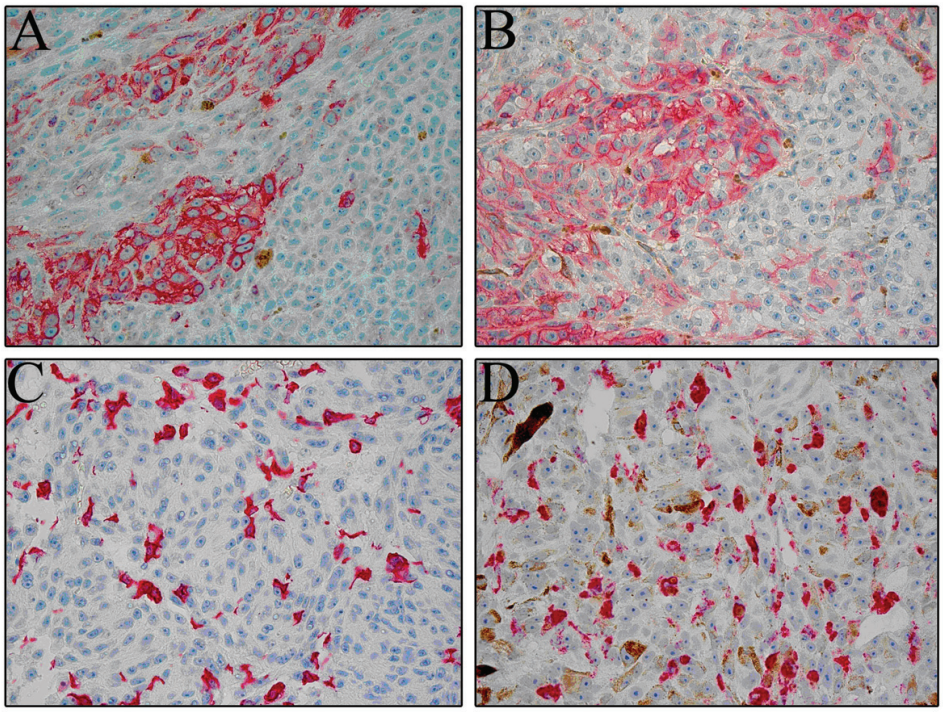


Fig. 3. HLA Class I and II expression and the presence of macrophages in uveal melanoma. **(A)** Uveal melanoma stained with mAb HC10 against HLA Class I. **(B)** and with mAb HCA2, also against HLA Class I. **(C)** mAb PG-M1 against macrophage epitope CD68 was used to label macrophages. **(D)** mAb clone Ta1.1B5 against HLA-DR. Magnification ×400. Reproduced with permission from *"Monosomy of chromosome 3 and an inflammatory phenotype occur together in uveal melanoma"*, by W. Maat, Invest. Ophthalmol. Vis. Sci. 2008, 49, 505–510, via Copyright Clearance Center.

do not carry a tolerogenic signal either. This needs further testing, but it is clear that uveal melanoma do not contain the kind of "patient friendly" immunostimulatory macrophages or dendritic cells that help stimulate immune responses against the tumor. In addition, not only local APC, but also factors secreted by the uveal melanoma cells or stromal cells within their microenvironment may have diverse effects, many of which remain to be fully characterised. Uveal melanoma cells for instance do express IDO, which may play a role in the creation of a local immune-privileged microenvironment [46]. Apart from blocking the effector function of the immune system, the local tumor environment may not only play a role in inhibiting effector T cells, but may also be involved at an earlier phase, i.e. in the abrogation of local antigen presentation [45]. Not only the cells that make up the tumor environment, but also cutaneous and uveal melanoma cells themselves are able to anergize the T cell-mediated immune response by alteration of dendritic cell function by tumor-derived cytokines. This process leads to the generation of suppressive and regulatory T cells, and this may play a role in immunotherapy of uveal melanoma [47].

3.3. The inflammatory phenotype

As mentioned above, De Waard-Siebinga et al. [36] observed a correlation between the density of macrophages and HLA Class I expression. Blom et al. [48], and later Ericsson et al. [49] and Dithmar et al. [50] reported that a higher expression of HLA class I antigens was associated with a worse survival. This may be related to the hematogeneous spreading of uveal melanoma: within the bloodstream, Natural Killer (NK) cells are able to kill tumor cells with a low HLA expression, while the NK cells would not kill circulating tumor cells with a high HLA Class I expression [51]. An increased expression of HLA Class II was also found to be associated with a worse survival [49]. HLA Class II antigens may be expressed on tumor cells as well as on infiltrating APCs. The density of TAM was compared to several other tumor characteristics (Table 1) [20]: a high TAM density was found to be associated with a range of prognostic markers, such as a largest basal tumor diameter ($P = 0.045$), the presence of monosomy 3 ($P = 0.001$), and a positive HLA Class I ($P = 0.017$) and HLA Class II ($P = 0.001$) staining (Fig. 3). This is the first study to find a significant association between macrophage density and monosomy 3. Using microarray-based gene-expression profiling, Onken et al. [52,53] identified two categories of tumors: Class 1 tumors are associated with the presence of spindle cells and carry an excellent prognosis, whereas class 2 tumors are associated with the presence of epithelioid cells, extravascular loops [54], monosomy 3, and metastatic death – a poor prognosis. Based on these associations, one can expect that the high risk class 2 tumors will be the ones with a high number of macrophages.

Table 1. Distribution of macrophage density in relation to clinical and histological parameters for antibody staining: numbers indicate mean and standard deviation for the concerning parameter. For other variables: number of cases with different macrophage densities. Cases were reported in Maat et al. [20].

		Macrophage density				
		N	**Low**	**Medium**	**High**	**p-value**
Gender	Male	27	13	18	6	0.03
	Female	23	3	12	8	
Cell type	Spindle	15	7	5	3	0.34
	Mixed + epithelioid	35	9	15	11	
CB involvement	Not present	29	11	11	7	0.55
	Present	21	5	9	7	
Tumor prominence	≤ 8.0 mm	31	10	13	8	0.98
	>8.0 mm	19	6	7	5	
Largest basal diameter	LBD ≤ 13.0 mm	29	13	8	8	0.05
	LBD >13.0 mm	21	3	12	6	
Chromosome 3	Disomy	19	12	5	2	0.001
	Monosomy	31	4	15	12	
Scleral ingrowth	Not present	3	3	0	0	0.03
	Present	47	13	20	14	
Loops	Not present	12	5	3	4	0.47
	Present	38	11	17	10	
Networks	Not present	19	8	5	6	0.28
	Present	31	8	15	8	
HC10		50	24.7 (28.2)	33.5 (27.9)	56.8 (35.3)	0.02
HCA2		50	36.3 (33.3)	41.0 (29.6)	54.6 (26.2)	0.23
Ta1.1B5		50	8.4 (5.4)	18.0 (18.6)	36.1 (29.4)	0.001

We proposed the term "inflammatory phenotype" to describe a combination of inflammatory markers in uveal melanoma: the most "ugly" tumors carry high numbers of macrophages and lymphocytes, high levels of HLA Class I and II expression, and are characterised by the presence of epithelioid cells and a high MVD. Several other inflammation-related molecules have been found in tumors with a bad prognosis, such as an increased expression of NFkB [55], COX-2 [56], of inducible Nitric Oxidase Synthase (iNOS) [57]. The presence of intra-tumoral FOXP3-positive regulatory T cells is correlated with the expression of COX-2 [35]. Whether the association with monosomy 3 determines the high grade of malignancy or indirectly induces local inflammation is as yet undetermined. Myeloid-Derived Suppressor Cells (MDSCs) are also induced by pro-inflammatory mediators, and enhance suppression of anti-tumor immune responses; the presence of regulatory T cells as well as MDSCs among the infiltrating tumor leukocytes would thus be consistent with inflammation being a "bad" prognostic factor for uveal melanoma and indeed being of functional relevance (further discussed below). The tumor-promoting M2 macrophages may be the same as MDSCs.

3.4. CD11b+ cells and T cell regulation

CD11b+ macrophages have been identified in uveal melanoma [36]. In recent years, many functions have been attributed to these CD11b+ macrophages, and they have been found to, for example, stimulate angiogenesis and lymphangiogenesis [58]. Furthermore, these cells not only occur in human uveal melanoma, but also infiltrate anterior chamber tumors in mice, where they have an immunological function and inhibit Cytotoxic T cell (CTL) activity[59]. CD11b+ macrophages can infiltrate tumors transplanted into the skin, where they inhibit T cell proliferation and induce T cell apoptosis via nitric oxide [60]. McKenna et al. [61] recently detected the presence of circulating CD11b+ cells in the blood of patients with uveal melanoma and compared their numbers to those in healthy controls. Using flow cytometry, the expression of CD15 (a marker for granulocytes) and CD68 was determined on CD11b+ myeloid cells. The percentage of CD11b+ cells was increased 1.8 fold in the blood of uveal melanoma patients compared to healthy controls. CD11b+ CD68–CD15+ cells (granulocytes) were increased 4.1 fold, while CD11b+ cells that expressed CD68 and low levels of CD15 (monocytes) were not significantly increased. At the same time, a reduction was seen in T cells expressing the CD3ζ chain, which indicates T cell dysfunction. The amount of T cell dysfunction correlated with the percentage of CD11b+ cells in the patient's blood. These findings are of great interest as CD15+ cells are able to stimulate the development of T regulator cells [62], which are able to suppress T cell function. Similar to the findings of De Waard-Siebinga et al. [36], McKenna's group showed that inside the uveal melanoma, CD11b+ cells express CD68, but not CD15 [61], indicating that these have macrophage and not granulocyte characteristics. Expression of the CD3ζ chain was reduced on tumor-infiltrating T cells in four out of five uveal melanomas studied, and it was suggested that CD68+ macrophages from the eye as well as the CD11b+ CD15+ granulocytes in the peripheral blood were able to reduce CD3ζ expression, either by increased arginase activity or by reactive oxygen production. From these data, peripheral blood CD11b+ cells may be assumed to belong to the MDSCs, while it is interesting that they are granulocytes. However, peripheral blood monocytes and granulocytes are known to share many lineage-associated antigens, such as the genetically polymorphic Human Monocyte Antigens 1 and 2 [63,64]. The immunosuppressive characteristics of these cells clearly indicate that they do not belong to the M1-type macrophages. Indeed, double-staining with anti-CD68 and anti-CD163 showed that most of the infiltrating cells in uveal melanoma are of the M2 type [65]: the majority of TAM was positive for both markers. As there is no specific marker for M1 macrophages, their absence could only be determined by exclusion.

4. Regulation of influx of macrophages

Macrophage infiltration is especially noticeable in a subset of uveal melanomas, i.e. tumors that contain epithelioid cells, are heavily pigmented

and contain a high microvascular density [12]. As discussed above, tumors that are characterised by an increased expression of HLA Class I, HLA Class II, and by infiltration with macrophages and lymphocytes are regarded as having an inflammatory phenotype [20]. Little is known about the biochemical pathways that stimulate the influx of lymphocytes and macrophages into uveal melanoma, but some general oncologic principles most likely apply to these tumors as well.

Monocytes tend to migrate to hypoxic areas, as hypoxia stimulates the stabilisation of HIF-1α, which stimulates the upregulation of many HIF-1α-dependent cytokines, including VEGF-A and -C, Placental Growth Factor (PlGF) and others [66]. These cytokines help to attract macrophages: in studies on ischemia-induced retinal neovascularisation in mice, VEGF was observed to play an important role in inducing leukocyte infiltration [67]. Monocyte/macrophage lineage cells express the VEGFR1 (flt1) receptor, through which VEGF exerts its chemotactic actions [68]. VEGF may also indirectly induce leukostasis and monocyte migration by increasing vascular permeability and increasing the expression of ICAM-1 [69]. Ischemia would thus create an environment that attracts macrophages and results in monocyte "trapping" [70]. Consistent with this, Mäkitie et al. [12] found an association between high levels of macrophages in uveal melanoma and a large tumor size, which was confirmed in a study on 50 uveal melanomas by Maat et al. [20] (Table 1). One assumes that the exponential growth of large tumors will induce ischemic areas, where upregulation of HIF-1α will occur, leading to the production of VEGF. This would then attract and trap more macrophages. It is therefore fascinating that Maat also found a correlation between monosomy 3 and an increased density of macrophages, most of which belong to the pro-angiogenic M2 type[65]. This may explain why tumors that have lost one chromosome 3 are more malignant. Larger tumors contain more areas with microvascular "hot spots" [71]. As macrophages have been hypothesized to be essential in the development of new vessels [72], the association between a high macrophage density and increased microvascular density sounds logical, and one can thus speculate that attraction of macrophages to ischemic areas may add to the effect of tumor tissue ischemia by causing a further production of VEGF. Macrophages would thus be the agents most involved in angiogenesis: blood vessels are essential features of malignancy of uveal melanoma.

Besides VEGF, a range of cytokines and chemokines may stimulate influx of macrophages including CCL2 (MCP-1) [73,74], CCL5, CCl7, CCL8, CXCL12, PDGF and M-CSF [75]. Gazzaniga et al. [76] demonstrated the importance of MCP-1 in tumor growth by transfecting a cutaneous melanoma cell line with the MCP-1 gene. After transfection, MCP-1 positive tumor cells grew faster in nude mice and became infiltrated by F4/80 cells, which marker identifies murine macrophages. Furthermore, MCP-1 positive tumors developed an extensive angiogenic network. Depletion of macrophages with clodronate liposomes resulted in 70% tumor growth inhibition, and fewer vessels. MCP-1 and macrophages were clearly shown to be important in tumor growth in this nude mouse model. However, we do not yet know all the chemokines and chemokine receptors that are being expressed in uveal melanoma. Repp et al. [77]

showed that uveal melanoma cells produce Macrophage-Migration-Inhibitory Factor (MIF). From the same group, Li et al.[78,79] examined the expression of CXCR4 and CCR7 on cell lines obtained from primary uveal melanoma and metastases, and found that both markers contributed to tumor-cell migration to the liver. Interestingly, liver extract was able to downregulate expression of these two chemokines on melanoma cells. Lowering the expression of CXCR4 on uveal melanoma cells with siRNA produced fewer liver metastases in a murine model. Expression of macrophage-specific chemokines in primary uveal melanoma and metastases warrants further investigation.
Aside from chemokines, cell-surface antigens may also play a role in macrophage migration. Mäkitie et al. [80] found expression of Ezrin, a protein that plays a role in cell migration, on uveal melanomas, and positive immunostaining of tumors was significantly associated with the presence of a high MVD and increased infiltration with macrophages. Clarijs et al. [81] studied the co-localization of macrophages with arcs, loops and network patterns [82], and analysed the potential involvement of endothelial monocyte-activating polypeptide (EMAP)-II in the process of macrophage infiltration in uveal melanomas using immunohistochemistry. They observed that macrophages had indeed accumulated in areas with a high EMAP-II expression, but that tumor cells and blood vessel endothelial cells in these areas also expressed ICAM-1. Vascular expression of ICAM-1 is also known to facilitate tissue infiltration of monocytes [83]. Ly et al. [84] observed that the aqueous humor of eyes enucleated for the presence of a uveal melanoma, frequently contained measurable levels of ICAM-1 and other adhesion molecules.
The association between the presence of macrophages and increased vascularity in uveal melanoma especially suggests an important role for M2 macrophages. A specific type of perivascular macrophage, carrying the TIE-2 receptor for angiopoietin, has been identified in a number of tumors[85].

5. Angiogenesis

5.1. Macrophages and angiogenesis

Experimental work has shown that macrophages play an important role in ischemia-induced pathological neovascularisation, for instance in the cornea: local depletion of macrophages around the cornea of mice leads to a complete inhibition of inflammatory corneal hem and angiogenesis[86]. Similarly, depletion of macrophages in an experimental mouse model for retinopathy of prematurity inhibited the development of pathological blood vessels [67]. Penfold et al. [87] suggested that choroidally-derived leukocytes and retinal microglia might be sources for signals leading to immune-mediated choroidal neovascularisation, and experimental murine models have been useful for studying the ocular location and function of different types of antigen-presenting cells [88]. One model developed to study the induction of ocular angiogenesis uses argon laser coagulation of the retina/choroid in mice in order to induce ischemia and subsequent hypoxia [89]. Eter et al. [90] studied the in

vivo contribution to angiogenesis of different types of mononuclear phagocytes (macrophages, dendritic cells), using mice that had been genetically modified so that their macrophages and dendritic cells became constitutively fluorescent (by expressing GFP, Green Fluorescent Protein). While normal retina already contained many GFP-positive fluorescent cells, 60 min after laser treatment, an intense accumulation of GFP-positive cells occurred around the laser-treated area. While dendritic cells disappeared rapidly, macrophages remained in situ for at least 7 weeks. Most of these cells carried the CD11b marker. Sakurai et al. [91] applied laser photocoagulation to the retina in 6–8 week old mice, and showed that macrophages stimulated angiogenesis, since macrophage depletion reduced choroidal neovascularisation. Itaya et al. [92] showed that both VEGF-A expression as well as macrophage infiltration could be blocked by intravitreal injection of a monoclonal antibody directed against monocyte chemoattractant protein (MCP-1). Immunohistochemical analysis showed that MCP-1 was strongly expressed in the RPE in areas that had been treated with laser, and this expression probably brought in VEGF-producing macrophages. Laser treatment stimulated the influx of M2-type macrophages, which are known to be involved in tissue repair as well as in angiogenesis.

5.2. Age and angiogenesis

Interestingly, wound healing studies have found a difference between young and old individuals with regard to angiogenesis. Old mice, for example, show an upregulation in angiogenesis during wound repair [93]. Espinosa-Heidmann et al.[94,95] and Apte et al. [96] showed that there was a differential influence of macrophages obtained from old and young mice. After laser treatment, laser spots in old mice contained more macrophages than laser spots in young mice, and developed much more choroidal angiogenesis; when macrophage depletion was performed prior to laser applications, the amount of blood vessels was lower. Splenic macrophages from young mice were able to inhibit angiogenesis in old mice, while an injection with macrophages from old mice did not inhibit angiogenesis [97]: macrophages from young mice had an anti-angiogenic influence, macrophages from old mice a pro-angiogenic one.
As blood vessel growth is important in tumor expansion, it was hypothesized that there might be a difference in the development of intraocular tumors in young and old mice [98]. Surprisingly, a recent study, using the B16 tumor-cell line in C57Bl/6 mice, showed no difference in tumor growth when cells were implanted into the anterior chamber of young or old mice [13]. However, once macrophages were removed at the time of tumor implantation, a dramatic difference ensued: tumors still grew in the anterior chamber of young mice, but hardly any tumor growth occurred in the anterior chamber of old mice. Not only the tumor-containing cells but also even the normal eyes of old mice expressed higher levels of macrophages and angiogenesis markers than the eyes of young mice. This corresponds to a report of a higher expression of many immunological markers in old mice compared to eyes of young mice, a phenomenon known as para-inflammation in the elderly [99]. We propose that the presence of increased numbers of intraocular macrophages in old age may

contribute to the development of specific age-related ocular diseases, including age-related macular degeneration as well as uveal melanoma. It is as yet unknown whether this is applied to all individuals or is determined by specific genetic or environmental determinants.
In studies by Kivelä's group, the presence of macrophages in human uveal melanoma has been found to correlate with MVD [42]. If the capacity of macrophages to stimulate or inhibit angiogenesis changes with age, a small dormant tumor might be helped to develop blood vessels when, during aging, the tumor-infiltrating macrophage population changes from an angiogenesis-inhibiting population to a pro-angiogenic one. Taken together, these observations suggest that the macrophages in close proximity to blood vessels and extravascular matrix networks in uveal melanoma are not just "innocent bystanders" but are actively involved in the growth and metastasis of uveal melanomas [81]. The pro-angiogenic capacity of macrophages thus makes it likely that their presence stimulates growth and dissemination of uveal melanoma and outgrowth of its metastases.

5.3. Uveal melanoma and matrix metalloproteinases

One important theory states that monocytes/macrophages are able to drill "tunnels" in existing tissues by using MMPs, and that these tunnels are later filled with new blood vessels [72]. Moldovan proposed that extracellular matrix is penetrated using macrophage-produced proteases, and then the empty spaces are colonized by either capillary sprouts, endothelial cells that are derived from trans-differentiated macrophages, or by circulating progenitor endothelial cells. Macrophages would thus be expected to be present at the tips of fast-growing capillaries. It is assumed that TAM in uveal melanoma expresses MMPs and function in this way. MMPs are probably involved in the pro-angiogenic function of macrophages in this tumor.

6. Macrophages in mice

6.1. Macrophages as scavengers

That macrophages are multifunctional and are not only active in angiogenesis is shown by different animal experiments, involving intraocular tumors. The translation of the word macrophage is "much-eater", and that is indeed one of its main functions: removal of debris by phagocytosis. In the eye, for example, this is seen during development with regression of the hyaloid vasculature including the pupillary membrane (located on the anterior surface of the lens) and the tunica vasculosa lentis (on the posterior surface of the lens), as well as the vitreal hyaloid vessels, which have typically disappeared at birth in humans [100]and postnatally in mice [101]. Macrophages are considered to play a role in programmed vascular regression: in PU.1 mice, which lack macrophages, vascular regression does not occur, and the different membranes and vessels remain [101]. Macrophages may achieve this effect by inducing apoptosis [102]. It may

well be that induction of apoptosis also plays a role in specific experimental ocular tumors, as shown in some tumor models.
Immune privilege of the eye allows the growth of tumor cells inside the eye while the same cells would be rejected when placed elsewhere in the body. In spite of this intraocular immune privilege, tumor rejection is still possible, and can follow several patterns [103]. In the first pattern, not only is the tumor destroyed, but the bystander tissues in the eye are also damaged, eventually leading to phthisis. This type of rejection depends on CD4+ cells and resembles a delayed-type hypersensitivity reaction. The second type of immune response depends on CD8+ cytotoxic T cells, leaving the eye intact. In addition, a third type of immune response has been identified in which the major cell type is the CD4+ T cell, but the eye is not destroyed [104,105].
Schurmans et al. [105] used a murine model that allowed visualisation of the tumor cells in the anterior chamber of the eye. Murine cells were transformed with the early region 1 of human adenovirus type 5 (Ad5E1) and when these cells were placed in the anterior chamber of a C57BL/6 mouse an intraocular tumor developed. After 3–4 weeks, the tumor disappeared without any damage to the neighbouring ocular tissues. Removal of the tumor cells is due to an immunological process, but rejection does not require TNFα, FasL, perforin, B cells, NK cells or CD8 cells. It does depend on CD4+ T cells, Interferon-gamma and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), as shown by Wang et al. [106].
As macrophages can do many different things, we analysed their role in the rejection process. On the one hand, macrophages and dendritic cells play an important role in antigen presentation to T cells and removal of macrophages should stop the development of an immune response. On the other hand, it is unlikely that tumor-specific CD4+ T cells can directly recognize and kill the Ad5E1 cells, as these are HLA Class II negative, while CD4+ T cells need Class II as a ligand. Perhaps the macrophages act as effector cells, killing the tumor cells or inducing apoptosis (a characteristic of the M1-type macrophages), or they may actively remove killed or damaged cells. Removal of effector macrophages would then prevent tumor-cell removal. This was tested in an experimental ocular tumor model, using adenovirus-transformed (Ad5E1) cells placed in the anterior chamber. Macrophages were removed from the eye using clodronate (dichloromethylene diphosphonate)-containing liposomes [107]. Clodronate is toxic for macrophages, macrophage precursors, as well as myeloid dendritic cells, and can be encased into liposomes, which are only taken up by macrophages; the release of clodronate inside the macrophage subsequently leads to macrophage apoptosis [108,109]. After tumor cell inoculation into the anterior chamber of the murine eye, clodronate-containing liposomes were injected subconjunctivally on several occasions. The intraocular tumors in mice that had undergone subconjunctival injections did not show any sign of rejection: the results were the opposite, namely that they showed a faster and more aggressive growth than the tumors in naïve mice or in control mice that received PBS-containing liposomes. While immunohistochemical staining identified F4/80 positive macrophages in draining lymph nodes of normal mice, no such cells were visible in tumor-containing eyes after

clodronate treatment. From this experiment, one can conclude that in this model, macrophages are essential in the destruction of this tumor. Their effect may be indirect: it may be that due to a lack of antigen presentation, effector T cells cannot develop; another option is that the macrophages are directly involved in cell killing: they kill the tumor cells and then remove them; and a third option is that macrophages are essential in directly helping the T cells in tumor-cell killing or in T cell induced apoptosis. Dace et al.[110] studied the role of macrophages in removal of intraocular tumors in immunodeficient mice. CD4+ T cells were isolated from a mouse that had just rejected an Ad5E1 tumor from its eye. Adoptive transfer of these CD4+ T lymphocytes into a severely immunodeficient (SCID) mouse protected these mice from intraocular tumor growth. However, when these SCID mice had been locally depleted of their ocular macrophages, they were no longer able to reject an intraocular tumor, even if they had received enough active CD4+ cells. These data indicate that, in this tumor-rejection model, CD4+ cells as well as macrophages are needed during the rejection process, and that macrophages actively contribute to the rejection itself. This fits with the findings of Boonman et al. [107] in the adenovirus-intraocular tumor model, where it was shown that depletion of macrophages led to progressive tumor growth within the eye. From these data we can learn that macrophages may be involved in rejecting intraocular tumors in this specific tumor model, for which reason macrophages should here be considered "friends" of the tumor-bearing host.

6.2. Immunostimulatory macrophages

Antigen-presenting cells are known to have different functions and depending on their activation state, they may suppress or stimulate immune responses. The observation that tumors can grow in the eye while they are immediately rejected when placed in the skin is considered an effect of the immune-privileged environment of the eye, in which antigen-presenting cells play an important role. Originally, it was proposed that antigens in the eye would not easily elicit an immune response due to the anatomical structure of the eye and the lack of intraocular lymph vessels. Due to the work of many and especially the group of J. Wayne Streilein, we now know that immune privilege in the eye is a consequence of many active immune processes, collectively known as Anterior Chamber Associated Immune Deviation, or ACAID. Placement of antigens in the anterior chamber of the eye leads to systemic tolerance. Eye-derived antigen-presenting cells (F4/80+ cells in mice) carry intraocular antigens to the spleen, where interactions with NK- (natural killer) T cells and marginal zone B cells help to create an environment where antigen-specific T cells develop into CD8+ regulatory T cells. These cells can downregulate CD4+ delayed-type hypersensitivity responses, as well as antibody responses and cytotoxic T cell responses [111]. In murine and human eyes, tumors can grow for a prolonged time before being recognized by the immune system or may not be attacked at all.
An experiment created by nature showed that this tolerance depends on the activation state of the local antigen-presenting cells and can be broken by

a spontaneous development of intense intra-tumoral inflammation. Mouse embryo cells transformed with Ad5E1A plus EJ-ras were placed into the anterior chamber of C57Bl/6 mice [112]. In general, E1A-transformed cells will grow in the anterior chamber and although specific CTLs can be found in the regional lymph nodes, they are not found systemically and tumor growth is not inhibited (Ad5E1A cells without EJ-ras grow more slowly and are eventually rejected, as shown by Schurmans et al.,[105]). However, in a later set of experiments using the Ad5E1A cells plus EJ-ras, over 50% of the mice developed another type of ocular disease: mice developed phthisis, a phenomenon where the tumor-containing eye shrinks [112]. Histological analysis showed a collapse of ocular integrity, filling of the anterior chamber with tumor, and shrinkage of the eye, with cellular infiltration of all ocular tissues. Mice that developed this type of ocular pathology also developed systemic tumor-specific CTL, most likely as a consequence of the phthisis.

As this was not seen in mice without phthisis, this suggested that the development of phthisis changed the ocular conditions that helped to induce CTL development. As we already know, regional lymph nodes were necessary to stimulate T cells, and local CD11c+ antigen-presenting cells were found to be essential in this process [105]. CD11c is expressed on some macrophages and especially on dendritic cells. There were important differences in the characteristics of the CD11c+ APCs from mice developing phthisis compared to mice with intraocular tumors but no phthisis: CD11c+ cells derived from the draining lymph nodes of mice with phthisis displayed upregulated cell-surface markers such as CD80, CD86, CD40 and MHC class I and II. The same was true for CD11c+ cells isolated from phthisical eyes containing an intraocular tumor. These markers were much less expressed on CD11c+ cells from lymph nodes of non-phthisical tumor-containing eyes.

The disintegration of the eye was accompanied by a local inflammatory response that produced APCs carrying an activated phenotype that helped to present tumor antigens, and stimulate systemic immune responses. The importance of such systemic immune responses was visible after a lethal Ad5E1A cell line was injected subcutaneously. While injection of this cell line is lethal in normal mice within a few weeks, all mice that had developed phthisis survived such an injection, and not even one of these mice developed subcutaneous tumors after being challenged. On the other hand, mice that had developed intraocular tumors without phthisis showed even growth of a subcutaneous non-aggressive Ad5 cell line that usually was rejected by naïve mice. This agrees with the phenomenon of ACAID, where placement of antigens into the anterior chamber induces systemic tolerance, not immunisation. The experiment that was provided to us by nature, i.e. the spontaneous development of phthisis in some mice with an intraocularly growing tumor, illustrates that the presence of activated antigen-presenting cells (dendritic cells and probably M1 macrophages) completely changes the outcome of an intraocular tumor and the effect of local immune responses [112]. In this case, the antigen-presenting cells can be regarded as “friends”, that helped to save the mouse’s life.

6.3. Immunoregulatory macrophages

While macrophages can be involved as scavengers and antigen-presenting cells, a specific immunoregulatory function has recently been ascribed to a subgroup of macrophages, i.e. the myeloid-derived suppressor cells (MDSCs). These cells can be induced by various tumour- and host-derived factors, including pro-inflammatory cytokines, and have been reported to accumulate in lymph nodes and in tumors in the majority of cancer patients and in experimental cancer models [113,114]. In the mouse, MDSCs are characterised by the following markers: CD11b+, Gr-1+, F4/80+, MHC class II low, Ly-6C+, CD31+[115,116]. These leukocytes include CD11b+ macrophages that have a tumor-stimulating role, as they help to induce immunosuppression and tolerance, and promote angiogenesis and metastasis. That MDSCs can be induced by pro-inflammatory cytokines supports the hypothesis that inflammation plays an important role in promoting tumor growth. Accumulation of leukocytes, including MDSCs, in lymph nodes and in a tumor may further enhance local inflammatory responses, and downregulate immune surveillance as well as anti-tumor immune responses. The potential role of MDSCs in inhibiting uveal melanoma immunotherapy requires further consideration.

MDSCs most probably have a physiological role in inflammation: they may be part of a feedback mechanism that prevents damage from overactive or prolonged inflammatory responses, and may help stop the expansion of CD8+ T cells. In normal tissues, a return to immune homeostasis may occur; however, in case of tumors, tumor cell escape may develop. It is not yet known whether the MDSCs are similar to the F4/80+ cells that are so important in murine experiments on ACAID.

CD11b+ macrophages have been attributed a role in T cell regulation, and McKenna and Kapp [59] showed that they are also involved in immune regulation against intraocular tumors in mice: CD11b+ macrophages can accumulate in intraocular murine tumors and suppress cytolytic anti-tumor CD8+ cells, thus allowing tumor growth. Such CD11b+ macrophages were able to inhibit CTL activity in vitro via NO production, and were also able to function in the skin, but inside the eye, macrophages showed a decreased capacity to produce NO [117]. This clearly shows the influence of the ocular microenvironment on the function of MDSCs.

A recent study with different mouse cancer cell lines showed that some highly malignant cell lines such as Lewis Lung Carcinoma can attract CD11b+/Gr+ inflammatory monocytes/myeloid suppressor cells and stimulate them to produce local TNFα [118]. TNFα subsequently enhanced metastatic growth of Lewis Lung Carcinoma cells. These observations clearly illustrate how tumors may use the innate immune system to support growth, and may also explain why an association may be seen between the presence of tumor-associated macrophages and poor patient prognosis. Obviously, these tumor-enhancing macrophages fall into the category of “foes” and not friends.

7. Implications for therapy

7.1 Macrophages

That macrophage depletion may prevent outgrowth of tumors was recently shown using a human melanoma xenograft model [76]. Data from our laboratory also show that this is the case when macrophage depletion is used in specific intraocular tumors [13,107]. However, Kelly et al. [97] observed a differential effect with regard to young and old mice: macrophages from young individuals were able to suppress the angiogenesis-stimulating effect of macrophages from older mice. As some diseases such as Age-related macular degeneration (AMD) and the growth of uveal melanomas are related to an older age, one can imagine that the lack of angiogenic inhibition by older macrophages may play a role in the development of these diseases. Ly et al. [13] observed an essential difference in the anterior segment of naïve eyes from young and old mice: eyes of old mice expressed more markers indicating the presence of M2 macrophages and blood vessels. The presence of more macrophages and blood vessels in aging eyes may contribute to the development of AMD as well as uveal melanoma, two diseases that are pathophysiologically related to vessel growth [87]. Whether environmental influences (food, smoking, drugs such as statins, aspirin and osteoporosis inhibitors) that may affect inflammation or macrophage activity or systemic conditions that involve inflammation (metabolic syndrome, auto-immune disease, increased acute phase proteins) play a role in the development of uveal melanoma or the outgrowth of metastases needs investigation.
As macrophages seem to be related to tumor growth, one would therefore like to either get rid of macrophages in the elderly, or otherwise change the functional characteristics of the macrophages. Would this be the fountain of youth? Transfusion of macrophages from genetically-identical individuals that differ in age is possible in inbred mice, but difficult in people: bone-marrow transplantation is quite a heavy treatment and probably not appropriate to treat uveal melanoma. It would be interesting to see whether a person's own macrophages can be modified in such a way as to reinstate a young phenotype with young-age-related functions. In vitro modification and re-infusion might then be an option to help suppress the development of vascularisation in malignancies. Anti-oxidants have been suggested as a potential treatment to modify the function of macrophages, and influencing macrophage function with exposure to different cytokines, similar to the treatments now used in cancer therapy, may also be an option [119]. In cutaneous melanoma for example, trials are underway to stimulate the cytotoxic function of macrophages by adding GM-CSF in combination with an anti-angiogenesis drug, thalidomide [120]. Depletion of the M2-type macrophages may at the same time enhance immune responsiveness, as M2-type macrophages are considered immunosuppressive. As there are two sides to the coin, one then wonders whether this approach would in fact induce more auto-immune disease. Another approach may be to inhibit the function of macrophages, for instance by blocking metalloproteinases, or by using macrophage-inhibiting drugs, such as biphosphonates [121].

A wide range of kinase inhibitors is currently being developed and used in the treatment of cancer. Kinases are also involved in the regulation of chemokines that may attract macrophages, such as macrophage inhibitory cytokine-1 [122]. In a first study we performed using kinase inhibitors in uveal melanoma, a differential effect between the response of primary tumors and metastases was found, with the metastases regrettably showing less sensitivity to the inhibitors than the primary tumors [123]. Further studies of the application of these interesting agents for uveal melanoma are very much indicated, and drugs that modify the pathway from ischemia to inflammation may be especially interesting.

7.2 Immunoregulation

As the presence of an inflammatory phenotype is associated with a bad prognosis in uveal melanoma, it may be worthwhile to identify ways to influence inflammation, which may also be involved in the outgrowth of liver metastases. Blocking inflammatory mediators, such as the NOS pathway by for instance non-steroidal anti-inflammatory drugs, may be a therapeutic option, although this has not been explored in uveal melanoma. In an animal model, targeted inhibition of inducible nitric oxide synthase was effective in inhibiting the growth of two different human cutaneous melanoma cell lines [124]. Furthermore, inflammation promotes the local accumulation of MDSCs, which are able to inhibit both the innate as well as the adaptive immune response [113]. MDSC's use inflammation-related enzymes such as ROS and iNOS2 to block T cell activity. 1-a25-dihydroxyvitamin D3 has been shown to inhibit GM-CSF induced formation of MDSCs [125] as does all-trans-retinoic acid [126]. As discussed earlier, the eyes of elder individuals show a generally increased expression of HLA Class II and signs of chronic inflammation. It may be important to try to find ways to downregulate this general phenomenon. The role of macrophage-suppressing drugs, such as biphosphonates, needs to be studied. Specifically attacking MDSCs may also be an option to suppress regulator T cells and thus support cytotoxic and delayed-type hypersensitivity T cells directed against tumor cells. The chemotherapeutic agent gemcitabine may specifically inhibit suppressor macrophages [127]. It is interesting that this drug has been found to have anti-tumor effects in vitro and is also mentioned in several clinical trials for uveal melanoma [128,129]. Another approach may be to stimulate anti-tumor immune responses so strongly that they can overcome suppression. One option for immunotherapy is stimulating anti-tumor immune responses with specific peptides, for which many approaches are feasible. When studying the possibility of combining adoptive T cell transfer with long peptide vaccination in a murine ocular tumor model, the vaccination stimulated the T cells so strongly, that they produced a deadly cytokine storm[130]. It will therefore be necessary to properly calibrate immune-stimulatory measures and to use the appropriate stimuli. Bosch and Ostrand-Rosenberg have used retroviruses to express HLA-DR and CD80 in tumor cells to activate tumor-specific CD4+ T lymphocytes [131,132]. Uveal melanoma cells were effectively transformed into a good vaccination source and were able to stimulate peripheral blood T cells

of uveal melanoma patients! As the presence of a uveal melanoma may induce MDSCs and a downregulated T cell activity, it is fascinating that with the proper stimulant, the inhibition of efficient T cell activity may be overcome.

8. Conclusions

Uveal melanoma is a deadly disease once metastases have developed and grown out. A wide range of prognostic parameters have been identified, one of which is the presence of an inflammatory phenotype, with increased tumor HLA expression and an increased presence of macrophages, as well as an upregulation of many pro-inflammatory molecules. The presence of macrophages may play an important pathophysiological role, especially as M2-type macrophages, the most prevalent type in uveal melanoma, has a tumor-promoting function. The reason that macrophages are important lies in their angiogenesis-promoting function, and their capacity to suppress anti-melanoma immune responses. The observations described in this review may help to extend the understanding of the diverse functions of macrophages and highlight their potential as "friends or foes" in human uveal melanoma. Finding ways to overcome the adverse effects that the presence of inflammation and macrophages has for patient survival, may help in developing new treatments to prevent the spread and growth of metastases in uveal melanoma.

Acknowledgements

We want to thank our collaborators in Leiden, especially Prof. Dr. G.P.M. Luyten, Dr. N.A. Gruis, Dr. P.A. van der Velden and Dr. Th. van Hall, and our students. We also want to thank the European Ophthalmic Oncology Group, for many useful meetings and comments. Dr. Jager is grateful to Prof. Dr. J.J. van Rood and Prof. Dr. R. van Furth for showing her the fun of monocytes and macrophages.

This work was supported by The Netherlands Organization for Scientific Research Mozaiek grant, The Dutch Cancer Foundation, Blindenhulp, Gratama Stichting, Landelijke Stichting voor Blinden en Slechtzienden, Leiden University Fund, Rotterdamse Vereniging Blindenbelangen, Stichting Blinden-Penning, Stichting Nederlands Oogheelkundig Onderzoek.

Abbreviations

ACAID	anterior chamber associated immune deviation
AMD	age-related macular degeneration
APC	antigen-presenting cell
COX-2	cyclo-oxygenase-2
CTL	cytotoxic T cell
DC	dendritic cell
ECM	extracellular matrix
EMAP	endothelial monocyte-activating polypeptide
EMMPRIN	extracellular MMP inducing protein
FGF	fibroblast growth factor
FNAB	fine needle aspiration biopsy
GFP	green fluorescent protein
GM-CSF	granulocyte-macrophage colony stimulating factor
HIF-1α	hypoxia-inducible factor 1alfa
HLA	human leukocyte antigen
HMA	human monocyte antigen
IDO	indolamine 2,3 dioxygenase
IFN	interferon
iNOS	inducible nitric oxidase synthase
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein
M-CSF	macrophage-colony stimulating factor
MDSC	myeloid-derived suppressor cells
MIC	macrophage inhibitory cytokine
MIF	macrophage-migration-inhibitory factor
MMP	matrix metalloproteinase
MVD	microvascular density
NFkB	nuclear factor kB
NK	natural killer
PAS	Periodic Acid Schiff
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
PlGF	placental growth factor
ROS	reactive oxygen species
RPE	retinal pigment epithelium
SCID	severe combined immunodeficiency
TAM	tumor-associated macrophages
TGFb	transforming growth factor beta
TIE-2	tyrosine kinase with immunoglobulin-like and IGF-like domains
TIMP	tissue inhibitor of MMPs
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
UV	ultraviolet
VEGF	vascular endothelial growth factor

References

1. Carr, I. *The macrophage. A review of ultrastructure and function.* Academic Press, New York. 1973.
2. Tauber, A. I. *Metchnikoff and the phagocytosis theory.* Nat.Rev.Mol.Cell Biol. 2003. 4:897-901.
3. van Furth, R., Cohn, Z. A., Hirsch, J. G., Humphrey, J. H., Spector, W. G., and Langevoort, H.L. *The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells.* Bull.World Health Organ 1972. 46:845-852.
4. Pollard, J. W. *Trophic macrophages in development and disease.* Nat.Rev.Immunol. 2009. 9:259-270.
5. McClellan, S. A., Huang, X., Barrett, R. P., van Rooijen, N., and Hazlett, L. D. *Macrophages restrict Pseudomonas aeruginosa growth, regulate polymorphonuclear neutrophil influx, and balance pro- and anti-inflammatory cytokines in BALB/c mice.* J Immunol. 2003. 170:5219-5227.
6. Kodukula, P., Liu, T., Rooijen, N. V., Jager, M. J., and Hendricks, R. L. *Macrophage control of herpes simplex virus type 1 replication in the peripheral nervous system.* J Immunol. 1999. 162:2895-2905.
7. van Klink, F., Taylor, W. M., Alizadeh, H., Jager, M. J., van Rooijen, N., and Niederkorn, J.Y.*The role of macrophages in Acanthamoeba keratitis.* Invest Ophthalmol Vis.Sci. 1996. 37:1271-1281.
8. Hu, J., Wang, Y., and Xie, L. *Potential role of macrophages in experimental keratomycosis.* Invest Ophthalmol Vis.Sci. 2009. 50:2087-2094.
9. Slegers, T. P., Broersma, L., van Rooijen, N., Hooymans, J. M., van Rij, G., and van der, G. R. *Macrophages play a role in the early phase of corneal allograft rejection in rats.* Transplantation 2004. 77:1641-1646.
10. Gabrilovich, D. I. and Nagaraj, S. *Myeloid-derived suppressor cells as regulators of the immune system.* Nat.Rev.Immunol. 2009. 9:162-174.
11. Kerr, E. C., Raveney, B. J., Copland, D. A., Dick, A. D., and Nicholson, L. B. *Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations.* J Autoimmun. 2008. 31:354-361.
12. Makitie, T., Summanen, P., Tarkkanen, A., and Kivela, T. *Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma.* Invest Ophthalmol Vis.Sci. 2001. 42:1414-1421.
13. Ly, L. V., Baghat, A., Versluis, M., Jordanova, E. S., Luyten, G. P., van Rooijen N., van Hall, T., van der Velden, and Jager, M. J. *In aged mice, outgrowth of intraocular melanoma depends on proangiogenic M2-type macrophages.* J.Immunol. 2010. 185:3481-3488.
14. Allavena, P., Sica, A., Garlanda, C., and Mantovani, A. *The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance.* Immunol.Rev. 2008. 222:155-161.
15. Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends Immunol. 2002. 23:549-555.
16. Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. *Cancer-related inflammation.* Nature 2008. 454:436-444.
17. Sica, A., Larghi, P., Mancino, A., Rubino, L., Porta, C., Totaro, M. G., Rimoldi, M., Biswas, S. K., Allavena, P., and Mantovani, A. *Macrophage polarization in tumour progression.* Semin. Cancer Biol. 2008. 18:349-355.
18. Lolmede, K., Campana, L., Vezzoli, M., Bosurgi, L., Tonlorenzi, R., Clementi, E., Bianchi, M.E., Cossu, G., Manfredi, A. A., Brunelli, S., and Rovere-Querini, P. *Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate H.* J Leukoc.Biol. 2009. 85:779-787.
19. Talmadge, J. E., Donkor, M., and Scholar, E. *Inflammatory cell infiltration of tumors: Jekyll or Hyde.* Cancer Metastasis Rev. 2007. 26:373-400.
20. Maat, W., Ly, L. V., Jordanova, E. S., Wolff-Rouendaal, D., Schalij-Delfos, N. E., and Jager, M. J. *Monosomy of chromosome 3 and an inflammatory phenotype occur together in uveal melanoma.* Invest Ophthalmol Vis.Sci. 2008. 49:505-510.
21. Bergman, L., Seregard, S., Nilsson, B., Ringborg, U., Lundell, G., and Ragnarsson-Olding, B. *Incidence of uveal melanoma in Sweden from 1960 to 1998.* Invest Ophthalmol Vis.Sci. 2002. 43:2579-2583.
22. Singh, A. D. and Topham, A. *Incidence of uveal melanoma in the United States: 1973-1997.*

Ophthalmology 2003. 110:956-961.
23. Kujala, E., Makitie, T., and Kivela, T. *Very long-term prognosis of patients with malignant uveal melanoma.* Invest Ophthalmol Vis.Sci. 2003. 44:4651-4659.
24. Ly, L. V., Odish, O. F., Wolff-Rouendaal, D., Missotten, G. S., Luyten, G. P., and Jager, M. J. *Intravascular presence of tumor cells as prognostic parameter in uveal melanoma: a 35-year survey.* Invest Ophthalmol.Vis.Sci. 2010. 51:658-665.
25. *Histopathologic characteristics of uveal melanomas in eyes enucleated from the Collaborative Ocular Melanoma Study. COMS report no. 6.* Am.J Ophthalmol 1998. 125:745-766.
26. Augsburger, J. J., Correa, Z. M., and Shaikh, A. H. *Effectiveness of treatments for metastatic uveal melanoma.* Am.J Ophthalmol 2009. 148:119-127.
27. Kodjikian, L., Grange, J. D., Baldo, S., Baillif, S., Garweg, J. G., and Rivoire, M. *Prognostic factors of liver metastases from uveal melanoma.* Graefes Arch.Clin Exp.Ophthalmol 2005. 243:985-993.
28. Gragoudas, E. S., Egan, K. M., Seddon, J. M., Glynn, R. J., Walsh, S. M., Finn, S. M., Munzenrider, J. E., and Spar, M. D. *Survival of patients with metastases from uveal melanoma.* Ophthalmology 1991. 98:383-389.
29. Mooy, C. M. and De Jong, P. T. *Prognostic parameters in uveal melanoma: a review.* Surv. Ophthalmol 1996. 41:215-228.
30. Mudhar, H. S., Parsons, M. A., Sisley, K., Rundle, P., Singh, A., and Rennie, I. G. *A critical appraisal of the prognostic and predictive factors for uveal malignant melanoma.* Histopathology 2004. 45:1-12.
31. Davidorf, F. H. and J. R. Lang. *Intraocular tumors*. 1977, pp. 119-133.
32. de la Cruz PO Jr, Specht, C. S., and McLean, I. W. *Lymphocytic infiltration in uveal malignant melanoma.* Cancer 1990. 65:112-115.
33. Vit, V. V. *Prognostic role of morphological characteristics of the immune response in uveal melanoblastomas of various cellular types.* Arkh.Patol. 1983. 45:25-30.
34. Whelchel, J. C., Farah, S. E., McLean, I. W., and Burnier, M. N. *Immunohistochemistry of infiltrating lymphocytes in uveal malignant melanoma.* Invest Ophthalmol Vis.Sci. 1993. 34:2603-2606.
35. Mougiakakos, D., Johansson, C. C., Trocme, E., ll-Ericsson, C., Economou, M. A., Larsson, O., Seregard, S., and Kiessling, R. *Intratumoral forkhead box P3-positive regulatory T cells predict poor survival in cyclooxygenase-2-positive uveal melanoma.* Cancer 2010. 116:2224-2233.
36. Waard-Siebinga, I., Hilders, C. G., Hansen, B. E., van Delft, J. L., and Jager, M. J. *HLA expression and tumor-infiltrating immune cells in uveal melanoma.* Graefes Arch.Clin Exp. Ophthalmol 1996. 234:34-42.
37. Schurmans, L. R., Blom, D. J., Waard-Siebinga, I., Keunen, J. E., Prause, J. U., and Jager, M. J. *Effects of transpupillary thermotherapy on immunological parameters and apoptosis in a case of primary uveal melanoma.* Melanoma Res. 1999. 9:297-302.
38. McLean, I. W., Foster, W. D., and Zimmerman, L. E. *Uveal melanoma: location, size, cell type, and enucleation as risk factors in metastasis.* Hum.Pathol. 1982. 13:123-132.
39. Woodward, J., Sisley, K., Reeves, G., Nichols, C., Parsons, M. A., Mudhar, H., and Rennie, I. *Evidence of macrophage and lymphocyte, but not dendritic cell, infiltration in posterior uveal melanomas, whilst cultured uveal melanomas demonstrate pluripotency by expressing CD68 and CD163.* Int.J.Exp.Pathol. 2004. 85:35-43.
40. Lau, S. K., Chu, P. G., and Weiss, L. M. *CD163: a specific marker of macrophages in paraffin-embedded tissue samples.* Am.J.Clin.Pathol. 2004. 122:794-801.
41. Nguyen, T. T., Schwartz, E. J., West, R. B., Warnke, R. A., Arber, D. A., and Natkunam, Y. *Expression of CD163 (hemoglobin scavenger receptor) in normal tissues, lymphomas, carcinomas, and sarcomas is largely restricted to the monocyte/macrophage lineage.* Am.J.Surg.Pathol. 2005. 29:617-624.
42. Toivonen, P., Makitie, T., Kujala, E., and Kivela, T. *Microcirculation and tumor-infiltrating macrophages in choroidal and ciliary body melanoma and corresponding metastases.* Invest Ophthalmol Vis.Sci. 2004. 45:1-6.
43. Torisu, H., Ono, M., Kiryu, H., Furue, M., Ohmoto, Y., Nakayama, J., Nishioka, Y., Sone, S., and Kuwano, M. *Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha.* Int.J Cancer 2000. 85:182-188.
44. Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., and Harris, A. L. *Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma.*

Cancer Res. 1996. 56:4625-4629.
45. Polak, M. E., Borthwick, N. J., Johnson, P., Hungerford, J. L., Higgins, B., Di Palma, S., Jager, M. J., and Cree, I. A. *Presence and phenotype of dendritic cells in uveal melanoma.* Br.J Ophthalmol 2007. 91:971-976.
46. Chen, P. W., Mellon, J. K., Mayhew, E., Wang, S., He, Y. G., Hogan, N., and Niederkorn, J.Y. *Uveal melanoma expression of indoleamine 2,3-deoxygenase: establishment of an immune privileged environment by tryptophan depletion.* Exp.Eye Res. 2007. 85:617-625.
47. Hallermalm, K., Seki, K., De, G. A., Motyka, B., Bleackley, R. C., Jager, M. J., Froelich, C. J., Kiessling, R., Levitsky, V., and Levitskaya, J. *Modulation of the tumor cell phenotype by IFN-gamma results in resistance of uveal melanoma cells to granule-mediated lysis by cytotoxic lymphocytes.* J.Immunol. 2008. 180:3766-3774.
48. Blom, D. J., Luyten, G. P., Mooy, C., Kerkvliet, S., Zwinderman, A. H., and Jager, M. J. *Human leukocyte antigen class I expression. Marker of poor prognosis in uveal melanoma.* Invest Ophthalmol Vis.Sci. 1997. 38:1865-1872.
49. Ericsson, C., Seregard, S., Bartolazzi, A., Levitskaya, E., Ferrone, S., Kiessling, R., and Larsson, O. *Association of HLA class I and class II antigen expression and mortality in uveal melanoma.* Invest Ophthalmol Vis.Sci. 2001. 42:2153-2156.
50. Dithmar, S., Crowder, J., Jager, M. J., Vigniswaran, N., and Grossniklaus, H. E. *HLA class I antigen expression correlates with histological cell type in uveal melanoma.* Ophthalmologe 2002. 99:625-628.
51. Jager, M. J., Hurks, H. M., Levitskaya, J., and Kiessling, R. *HLA expression in uveal melanoma: there is no rule without some exception.* Hum.Immunol. 2002. 63:444-451.
52. Onken, M. D., Worley, L. A., Ehlers, J. P., and Harbour, J. W. *Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death.* Cancer Res. 2004. 64:7205-7209.
53. Onken, M. D., Lin, A. Y., Worley, L. A., Folberg, R., and Harbour, J. W. *Association between microarray gene expression signature and extravascular matrix patterns in primary uveal melanomas.* Am.J Ophthalmol 2005. 140:748-749.
54. Chang, S. H., Worley, L. A., Onken, M. D., and Harbour, J. W. *Prognostic biomarkers in uveal melanoma: evidence for a stem cell-like phenotype associated with metastasis.* Melanoma Res. 2008. 18:191-200.
55. Dror, R., Lederman, M., Umezawa, K., Barak, V., Pe'er, J., and Chowers, I. *Characterizing the Involvement of the Nuclear Factor-kappa B (NF{kappa}B) transcription factor in uveal melanoma.* Invest Ophthalmol Vis.Sci. 2010. 51:1811-1816.
56. Figueiredo, A., Caissie, A. L., Callejo, S. A., McLean, I. W., Gold, P., and Burnier, M. N., Jr. *Cyclooxygenase-2 expression in uveal melanoma: novel classification of mixed-cell-type tumours.* Can.J Ophthalmol 2003. 38:352-356.
57. Johansson, C. C., Mougiakakos, D., Trocme, E., All-Ericsson, C., Economou, M. A., Larsson, O., Seregard, S., and Kiessling, R. *Expression and prognostic significance of iNOS in uveal melanoma.* Int.J Cancer 2010. 126:2682-2689.
58. Maruyama, K., Ii, M., Cursiefen, C., Jackson, D. G., Keino, H., Tomita, M., van Rooijen, N., Takenaka, H., D'Amore, P. A., Stein-Streilein, J., Losordo, D. W., and Streilein, J. W. *Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages.* J Clin Invest 2005. 115:2363-2372.
59. McKenna, K. C. and Kapp, J. A. *Accumulation of immunosuppressive CD11b+ myeloid cells correlates with the failure to prevent tumor growth in the anterior chamber of the eye.* J Immunol. 2006. 177:1599-1608.
60. Saio, M., Radoja, S., Marino, M., and Frey, A. B. *Tumor-infiltrating macrophages induce apoptosis in activated CD8(+) T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide.* J Immunol. 2001. 167:5583-5593.
61. McKenna, K. C., Beatty, K. M., Bilonick, R. A., Schoenfield, L., Lathrop, K. L., and Singh, A. D. *Activated CD11b+ CD15+ granulocytes increase in the blood of patients with uveal melanoma.* Invest Ophthalmol Vis.Sci. 2009. 50:4295-4303.
62. Huang, B., Pan, P. Y., Li, Q., Sato, A. I., Levy, D. E., Bromberg, J., Divino, C. M., and Chen, S. H. *Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host.* Cancer Res. 2006. 66:1123-1131.
63. Jager, M. J., Claas, F. H., Witvliet, M., and van Rood, J. J. *Correspondence of the monocyte antigen HMA-1 to the non-HLA antigen 9a.* Immunogenetics 1986. 23:71-77.
64. Jager, M. J., Claas, F. H., D'Amaro, J., Baldwin, W. M., and van Rood, J. J. *Two alloantigens on*

human monocytes: a diallelic system? Hum.Immunol. 1987. 19:215-224.
65. Bronkhorst, I. H., Ly, L. V., Jordanova, E. S., Vrolijk, H., Versluis, M., Luyten, G. P., and Jager, M. J. *Detection of M2 macrophages in uveal melanoma and relation with survival.* Invest Ophthalmol.Vis.Sci. 2010.In Press.
66. Campochiaro, P. A. *Ocular versus extraocular neovascularization: mirror images or vague resemblances.* Invest Ophthalmol Vis.Sci. 2006. 47:462-474.
67. Ishida, S., Usui, T., Yamashiro, K., Kaji, Y., Amano, S., Ogura, Y., Hida, T., Oguchi, Y., Ambati, J., Miller, J. W., Gragoudas, E. S., Ng, Y. S., D'Amore, P. A., Shima, D. T., and Adamis, A. P. *VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization.* J Exp.Med. 2003. 198:483-489.
68. Barleon, B., Sozzani, S., Zhou, D., Weich, H. A., Mantovani, A., and Marme, D. *Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1.* Blood 1996. 87:3336-3343.
69. Miyamoto, K., Khosrof, S., Bursell, S. E., Moromizato, Y., Aiello, L. P., Ogura, Y., and Adamis, A. P. *Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1).* Am.J Pathol. 2000. 156:1733-1739.
70. Bosco, M. C., Puppo, M., Blengio, F., Fraone, T., Cappello, P., Giovarelli, M., and Varesio, L. *Monocytes and dendritic cells in a hypoxic environment: Spotlights on chemotaxis and migration.* Immunobiology 2008. 213:733-749.
71. Makitie, T., Summanen, P., Tarkkanen, A., and Kivela, T. *Microvascular density in predicting survival of patients with choroidal and ciliary body melanoma.* Invest Ophthalmol Vis.Sci. 1999. 40:2471-2480.
72. Moldovan, N. I. *Role of monocytes and macrophages in adult angiogenesis: a light at the tunnel's end.* J Hematother.Stem Cell Res. 2002. 11:179-194.
73. Gabrilovich, D. *Mechanisms and functional significance of tumour-induced dendritic-cell defects.* Nat.Rev.Immunol. 2004. 4:941-952.
74. Huang, B., Lei, Z., Zhao, J., Gong, W., Liu, J., Chen, Z., Liu, Y., Li, D., Yuan, Y., Zhang, G. M., and Feng, Z. H. *CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers.* Cancer Lett. 2007. 252:86-92.
75. Sica, A., Allavena, P., and Mantovani, A. *Cancer related inflammation: the macrophage connection.* Cancer Lett. 2008. 267:204-215.
76. Gazzaniga, S., Bravo, A. I., Guglielmotti, A., van Rooijen, N., Maschi, F., Vecchi, A., Mantovani, A., Mordoh, J., and Wainstok, R. *Targeting tumor-associated macrophages and inhibition of MCP-1 reduce angiogenesis and tumor growth in a human melanoma xenograft.* J Invest Dermatol. 2007. 127:2031-2041.
77. Repp, A. C., Mayhew, E. S., Apte, S., and Niederkorn, J. Y. *Human uveal melanoma cells produce macrophage migration-inhibitory factor to prevent lysis by NK cells.* J Immunol. 2000. 165:710-715.
78. Li, H., Alizadeh, H., and Niederkorn, J. Y. *Differential expression of chemokine receptors on uveal melanoma cells and their metastases.* Invest Ophthalmol Vis.Sci. 2008. 49:636-643.
79. Li, H., Yang, W., Chen, P. W., Alizadeh, H., and Niederkorn, J. Y. *Inhibition of chemokine receptor expression on uveal melanomas by CXCR4 siRNA and its effect on uveal melanoma liver metastases.* Invest Ophthalmol Vis.Sci. 2009. 50:5522-5528.
80. Makitie, T., Carpen, O., Vaheri, A., and Kivela, T. *Ezrin as a prognostic indicator and its relationship to tumor characteristics in uveal malignant melanoma.* Invest Ophthalmol Vis.Sci. 2001. 42:2442-2449.
81. Clarijs, R., Schalkwijk, L., Ruiter, D. J., and de Waal, R. M. *EMAP-II expression is associated with macrophage accumulation in primary uveal melanoma.* Invest Ophthalmol Vis.Sci. 2003. 44:1801-1806.
82. Folberg, R., Hendrix, M. J., and Maniotis, A. J. *Vasculogenic mimicry and tumor angiogenesis.* Am.J Pathol. 2000. 156:361-381.
83. Bevilacqua, M. P. *Endothelial-leukocyte adhesion molecules.* Annu.Rev.Immunol. 1993. 11:767-804.
84. Ly, L. V., Bronkhorst, I. H., van, B. E., Vrolijk, J., Taylor, A. W., Versluis, M., Luyten, G. P., and Jager, M. J. *Inflammatory cytokines in eyes with uveal melanoma and relation with macrophage infiltration.* Invest Ophthalmol.Vis.Sci. 2010. 51:5445-5451.
85. Venneri, M. A., De Palma, M., Ponzoni, M., Pucci, F., Scielzo, C., Zonari, E., Mazzieri, R., Doglioni, C., and Naldini, L. *Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer.* Blood 2007. 109:5276-5285.

86. Cursiefen, C., Chen, L., Borges, L. P., Jackson, D., Cao, J., Radziejewski, C., D'Amore, P. A., Dana, M. R., Wiegand, S. J., and Streilein, J. W. *VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment.* J Clin Invest 2004.113:1040-1050.
87. Penfold, P. L., Madigan, M. C., Gillies, M. C., and Provis, J. M. *Immunological and aetiological aspects of macular degeneration.* Prog.Retin.Eye Res. 2001. 20:385-414.
88. McMenamin, P. G. *Dendritic cells and macrophages in the uveal tract of the normal mouse eye.* Br.J Ophthalmol 1999. 83:598-604.
89. Ding, X., Patel, M., and Chan, C. C. *Molecular pathology of age-related macular degeneration.* Prog.Retin.Eye Res. 2009. 28:1-18.
90. Eter, N., Engel, D. R., Meyer, L., Helb, H. M., Roth, F., Maurer, J., Holz, F. G., and Kurts, C. *In vivo visualization of dendritic cells, macrophages, and microglial cells responding to laser-induced damage in the fundus of the eye.* Invest Ophthalmol Vis.Sci. 2008. 49:3649-3658.
91. Sakurai, E., Anand, A., Ambati, B. K., van Rooijen, N., and Ambati, J. *Macrophage depletion inhibits experimental choroidal neovascularization.* Invest Ophthalmol Vis.Sci. 2003. 44:3578-3585.
92. Itaya, M., Sakurai, E., Nozaki, M., Yamada, K., Yamasaki, S., Asai, K., and Ogura, Y. *Upregulation of VEGF in murine retina via monocyte recruitment after retinal scatter laser photocoagulation.* Invest Ophthalmol Vis.Sci. 2007. 48:5677-5683.
93. Ashcroft, G. S., Horan, M. A., and Ferguson, M. W. *Aging is associated with reduced deposition of specific extracellular matrix components, an upregulation of angiogenesis, and an altered inflammatory response in a murine incisional wound healing model.* J Invest Dermatol. 1997. 108:430-437.
94. Espinosa-Heidmann, D. G., Suner, I., Hernandez, E. P., Frazier, W. D., Csaky, K. G., and Cousins, S. W. *Age as an independent risk factor for severity of experimental choroidal neovascularization.* Invest Ophthalmol Vis.Sci. 2002. 43:1567-1573.
95. Espinosa-Heidmann, D. G., Suner, I. J., Hernandez, E. P., Monroy, D., Csaky, K. G., and Cousins, S. W. *Macrophage depletion diminishes lesion size and severity in experimental choroidal neovascularization.* Invest Ophthalmol Vis.Sci. 2003. 44:3586-3592.
96. Apte, R. S., Richter, J., Herndon, J., and Ferguson, T. A. *Macrophages inhibit neovascularization in a murine model of age-related macular degeneration.* PLoS Med. 2006. 3:e310.
97. Kelly, J., Ali, K. A., Yin, J., Ferguson, T. A., and Apte, R. S. *Senescence regulates macrophage activation and angiogenic fate at sites of tissue injury in mice.* J Clin Invest 2007. 117:3421-3426.
98. Jager, M. J. and Klaver, C. C. *Macrophages feel their age in macular degeneration.* J Clin Invest 2007. 117:3182-3184.
99. Xu, H., Chen, M., and Forrester, J. V. *Para-inflammation in the aging retina.* Prog.Retin.Eye Res. 2009. 28:348-368.
100. Zhu, M., Madigan, M. C., van Driel, D., Maslim, J., Billson, F. A., Provis, J. M., and Penfold, P. L. *The human hyaloid system: cell death and vascular regression.* Exp.Eye Res. 2000. 70:767-776.
101. Lang, R. A. and Bishop, J. M. *Macrophages are required for cell death and tissue remodeling in the developing mouse eye.* Cell 1993. 74:453-462.
102. Diez-Roux, G. and Lang, R. A. *Macrophages induce apoptosis in normal cells in vivo.* Development 1997. 124:3633-3638.
103. Knisely, T. L., Luckenbach, M. W., Fischer, B. J., and Niederkorn, J. Y. *Destructive and nondestructive patterns of immune rejection of syngeneic intraocular tumors.* J Immunol. 1987. 138:4515-4523.
104. Niederkorn, J. Y. *Immune escape mechanisms of intraocular tumors.* Prog.Retin.Eye Res. 2009. 28:329-347.
105. Schurmans, L. R., Diehl, L., den Boer, A. T., Sutmuller, R. P., Boonman, Z. F., Medema, J. P., van der Voort, E. I., Laman, J., Melief, C. J., Jager, M. J., and Toes, R. E. *Rejection of intraocular tumors by CD4(+) T cells without induction of phthisis.* J Immunol. 2001. 167:5832-5837.
106. Wang, S., Boonman, Z. F., Li, H. C., He, Y., Jager, M. J., Toes, R. E., and Niederkorn, J. Y. *Role of TRAIL and IFN-gamma in CD4+ T cell-dependent tumor rejection in the anterior chamber of the eye.* J Immunol. 2003. 171:2789-2796.
107. Boonman, Z. F., Schurmans, L. R., van Rooijen, N., Melief, C. J., Toes, R. E., and Jager, M. J. *Macrophages are vital in spontaneous intraocular tumor eradication.* Invest Ophthalmol Vis.Sci. 2006. 47:2959-2965.
108. van Rooijen, N. and Sanders, A. *Liposome mediated depletion of macrophages: mechanism of*

action, preparation of liposomes and applications. J Immunol.Methods 1994. 174:83-93.
109. van Rooijen, N., Sanders, A., and van den Berg, T. K. *Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine.* J Immunol.Methods 1996. 193:93-99.
110. Dace, D. S., Chen, P. W., and Niederkorn, J. Y. *CD4+ T-cell-dependent tumour rejection in an immune-privileged environment requires macrophages.* Immunology 2008. 123:367-377.
111. Streilein, J. W., Masli, S., Takeuchi, M., and Kezuka, T. *The eye's view of antigen presentation.* Hum.Immunol. 2002. 63:435-443.
112. Boonman, Z. F., van Mierlo, G. J., Fransen, M. F., de Keizer, R. J., Jager, M. J., Melief, C. J., and Toes, R. E. *Maintenance of immune tolerance depends on normal tissue homeostasis.* J Immunol. 2005. 175:4247-4254.
113. Ostrand-Rosenberg, S. and Sinha, P. *Myeloid-derived suppressor cells: linking inflammation and cancer.* J Immunol. 2009. 182:4499-4506.
114. Sinha, P., Clements, V. K., Bunt, S. K., Albelda, S. M., and Ostrand-Rosenberg, S. *Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response.* J Immunol. 2007. 179:977-983.
115. Dolcetti, L., Marigo, I., Mantelli, B., Peranzoni, E., Zanovello, P., and Bronte, V. *Myeloid-derived suppressor cell role in tumor-related inflammation.* Cancer Lett. 2008. 267:216-225.
116. Marigo, I., Dolcetti, L., Serafini, P., Zanovello, P., and Bronte, V. *Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells.* Immunol.Rev. 2008. 222:162-179.
117. Miguel, R. D., Cherpes, T. L., Watson, L. J., and McKenna, K. C. *CTL induction of tumoricidal nitric oxide production by intratumoral macrophages is critical for tumor elimination.* J.Immunol. 2010. 185:6706-6718.
118. Kim, S., Takahashi, H., Lin, W. W., Descargues, P., Grivennikov, S., Kim, Y., Luo, J. L., and Karin, M. *Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis.* Nature 2009. 457:102-106.
119. Dace, D. S., Khan, A. A., Kelly, J., and Apte, R. S. *Interleukin-10 promotes pathological angiogenesis by regulating macrophage response to hypoxia during development.* PLoS One. 2008. 3:e3381.
120. Lutzky, J., Weber, R., Nunez, Y., Gillett, M., and Spitler, L. *A phase 1 study of granulocyte macrophage colony-stimulating factor (sargramostim) and escalating doses of thalidomide in patients with high-risk malignant melanoma.* J Immunother. 2009. 32:79-85.
121. de Vries. E., van der Weij, J. P., van, d., V, van Paassen, H. C., Jager, M. J., Sleeboom, H. P., Bijvoet, O. L., and Cats, A. *In vitro effect of (3-amino-1-hydroxypropylidene)-1,1-bisphosphonic acid (APD) on the function of mononuclear phagocytes in lymphocyte proliferation.* Immunology 1982. 47:157-163.
122. Boyle, G. M., Pedley, J., Martyn, A. C., Banducci, K. J., Strutton, G. M., Brown, D. A., Breit, S. N., and Parsons, P. G. *Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity.* J Invest Dermatol. 2009. 129:383-391.
123. Maat, W., el Filali, M., Dirks-Mulder, A., Luyten, G. P., Gruis, N. A., Desjardins, L., Boender, P., Jager, M. J., and van der Velden, P. A. *Episodic Src activation in uveal melanoma revealed by kinase activity profiling.* Br.J Cancer 2009. 101:312-319.
124. Sikora, A. G., Gelbard, A., Davies, M. A., Sano, D., Ekmekcioglu, S., Kwon, J., Hailemichael, Y., Jayaraman, P., Myers, J. N., Grimm, E. A., and Overwijk, W. W. *Targeted inhibition of inducible nitric oxide synthase inhibits growth of human melanoma in vivo and synergizes with chemotherapy.* Clin.Cancer Res. 2010. 16:1834-1844.
125. Young, M. R., Halpin, J., Hussain, R., Lozano, Y., Djordjevic, A., Devata, S., Matthews, J. P., and Wright, M. A. *Inhibition of tumor production of granulocyte-macrophage colony-stimulating factor by 1 alpha, 25-dihydroxyvitamin D3 reduces tumor motility and metastasis.* Invasion Metastasis 1993. 13:169-177.
126. Kusmartsev, S., Cheng, F., Yu, B., Nefedova, Y., Sotomayor, E., Lush, R., and Gabrilovich, D. *All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination.* Cancer Res. 2003. 63:4441-4449.
127. Suzuki, E., Kapoor, V., Jassar, A. S., Kaiser, L. R., and Albelda, S. M. *Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity.* Clin Cancer Res. 2005. 11:6713-6721.
128. Corrie, P. G., Shaw, J., Spanswick, V. J., Sehmbi, R., Jonson, A., Mayer, A., Bulusu, R., Hartley, J. A., and Cree, I. A. *Phase I trial combining gemcitabine and treosulfan in advanced cutaneous and uveal melanoma patients.* Br.J Cancer 2005. 92:1997-2003.

129. Neale, M. H., Myatt, N. E., Khoury, G. G., Weaver, P., Lamont, A., Hungerford, J. L.,Kurbacher, C. M., Hall, P., Corrie, P. G., and Cree, I. A. *Comparison of the ex vivo chemosensitivity of uveal and cutaneous melanoma.* Melanoma Res. 2001. 11:601-609.
130. Ly, L. V., Sluijter, M., Versluis, M., Luyten, G. P., van der Burg, S. H., Melief, C. J., Jager, M. J., and van Hall T. *Peptide vaccination after T-cell transfer causes massive clonal expansion, tumor eradication, and manageable cytokine storm.* Cancer Res. 2010. 70:8339-8346.
131. Bosch, J. J., Thompson, J. A., Srivastava, M. K., Iheagwara, U. K., Murray, T. G., Lotem, M., Ksander, B. R., and Ostrand-Rosenberg, S. *MHC class II-transduced tumor cells originating in the immune-privileged eye prime and boost CD4(+) T lymphocytes that cross-react with primary and metastatic uveal melanoma cells.* Cancer Res. 2007. 67:4499-4506.
132. Dissanayake, S. K., Thompson, J. A., Bosch, J. J., Clements, V. K., Chen, P. W., Ksander, B. R., and Ostrand-Rosenberg, S. *Activation of tumor-specific CD4(+) T lymphocytes by major histocompatibility complex class II tumor cell vaccines: a novel cell-based immunotherapy.* Cancer Res. 2004. 64:1867-1874.