ORIGINAL ARTICLES – Laboratory science

Macrophages in proliferative vitreoretinopathy and proliferative diabetic retinopathy: differentiation of subpopulations

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Abstract

Macrophages have long been known to play a major role in the pathogenesis of proliferative vitreoretinal disorders. Using the monoclonal antibodies EBM11 (pan macrophage), 27E10 (early inflammatory stage marker), and RM3/1 (healing phase marker), different subpopulations of macrophages were differentiated in surgically removed membranes from patients with macular pucker (n=6), proliferative vitreoretinopathy (PVR) following rhegmatogenous retinal detachment (n=11), traumatic PVR (n=19), and proliferative diabetic retinopathy (PDR) (n=11). Macrophages were predominantly found in traumatic PVR and PDR. Some healing phase (RM3/1) macrophages were detected in all disease entities. Inflammatory stage macrophages (positive staining for 27E10) could not be detected in PVR following rhegmatogenous retinal detachment and idiopathic macular pucker. In traumatic PVR inflammatory stage macrophages were associated with a short history of disease whereas in PDR all types of macrophages could be detected regardless of clinical history and duration of the disease.

(*Br J Ophthalmol* 1993; 77: 731–733)

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Accepted for publication 13 July 1993

Occurrence of macrophages in epiretinal membranes from patients with proliferative vitreoretinopathy is a long known fact that has been documented by morphological studies[1] and immunostaining.[2] Experimental data indicate the involvement of macrophages in the pathogenesis of proliferative vitreoretinopathy (PVR).[3,4] However, macrophages have yet to be identified immunohistochemically in tissue derived from patients with proliferative diabetic retinopathy (PDR) and macular pucker.

Since PVR is a multistage disease process with a great variety in the time course of progression to traction retinal detachment, the search for specific markers of disease severity and inflammatory stages seems to be vital for a better understanding of PVR. In the following study we report the different prevalence of two macrophage markers, 27E10 (an early inflammatory stage marker) and RM3/1 (a healing phase marker), in different proliferative vitreoretinal disease entities and correlate our findings with clinical history and duration of the disease process.

Material and methods

Epiretinal membranes were obtained from patients undergoing vitrectomy for a variety of vitreoretinal disorders. The membranes were immediately frozen at −70°C and cut on a cryostat with subsequent acetone fixation for 10 minutes.

The general immunostaining procedures were performed as described previously.[5] Antibodies were diluted in phosphate buffered saline containing 0·5% bovine serum albumin. The following mouse derived antibodies and dilutions were used:

Anti RM3/1 (healing phase macrophage), Biomedicals AG	1:20
Anti 27E10 (inflammatory phase macrophage), Biomedicals AG	1:20
Anti EBM11 (pan-macrophage), Dakopatts	1:20

The primary antibodies were labelled with a biotin-streptavidin system.

Antimouse-biotin (Dakopatts)	1:400
Streptavidin-alkaline phosphatase (Dakopatts)	1:1000

Alkaline phosphatase was visualised using the fast red stain. Counterstaining was performed with haematoxylin and eosin. Negative controls were performed by exchanging the primary antibody with mouse IgG.

Results

In most of the diabetic membranes (10/11) macrophages could be detected by the pan macrophage antibody EBM11 regardless of duration or

Table 1 Proliferative diabetic retinopathy

No	EMB11 (pan macrophage)	27E10 (inflammatory phase macrophage)	RM3/1 (healing phase macrophage)	Type of diabetes	Duration LC of diabetes (years)	LC	PPV
1	++	+	+	II	3	y	n
2	+	(+)	+	I	4	y	y
3	++	++	−	II	4	y	n
4	+	−	+	II	10	n	n
5	++	+	(+)	II	15	y	y
6	−	−	−	I	15	y	n
7	+	+	+	II	17	y	y
8	++	+	+	I	18	y	n
9	+	−	+	II	20	n	n
10	++	+	(+)	II	21	y	y
11	+	−	−	I	30	y	n

Staining intensity: ++=Large number of macrophages detected throughout the membrane. +=Few macrophages scattered over the membrane or cluster of macrophages. (+)=Single macrophage. −=No staining detected.
Duration=duration of diabetes before operation in years. Type=diabetes type I, diabetes type II. LC=previous laser coagulation (y=yes, n=no). PPV=previous surgical intervention (y=yes, n=no).

type of diabetes. In many cases (5/11) macrophages could be detected in large numbers scattered throughout the membranes. Inflammatory stage macrophages (7/11) and healing phase macrophages (8/11) were identified in several membranes. However, no correlation between type of diabetes or medical history and the appearance of a certain subtype of macrophage could be detected (Table 1).

In PVR following rhegmatogenous retinal detachment with scleral buckling procedures and in idiopathic macular pucker, no inflammatory stage macrophages (27E10) could be detected. Only in three cases of PVR after rhegmatogenous retinal detachment with the shortest history of progression from scleral buckling procedures to epiretinal proliferation and in one case of idiopathic macular pucker was positive labelling of macrophages with RM3/1 observed (Tables 2 and 3).

In traumatic PVR most of the membranes (12/19) reacted positively with the pan macrophage antibody EMB11. Interestingly, most of the patients with positive labelling of EMB11 (pan macrophage) and 27/E10 (inflammatory macrophage) had a medical history of less than 9 months between traumatic event or surgical intervention and traction retinal detachment caused by PVR. Only seven cases demonstrated positive labelling for the healing phase macrophage (RM3/1). In these patients, retinal surgery had been performed between 4 and 24 months after the initial traumatic event. In six membranes of traumatic origin, healing phase and inflammatory phase macrophages could be localised in parallel sections. We could not detect any connection between the type of previous surgery and the occurrence of a certain subtype of macrophages (Table 4).

Table 2 Proliferative vitreoretinopathy following rhegmatogenous retinal detachment

No	EMB11 (pan macrophage)	27E10 (inflammatory phase macrophage)	RM3/1 (healing phase macrophage)	Duration (months)
1	(+)	–	(+)	4
2	+	–	+	5
3	+	–	(+)	7
4	–	–	–	8
5	–	–	–	12
6	+	–	–	24
7	–	–	–	25
8	–	–	–	26
9	(+)	–	–	26
10	–	–	–	30
11	–	–	–	98

Staining intensity: + = Few macrophages scattered over the membrane or cluster of macrophages. (+) = Single macrophage. – = No staining detected.
Duration = months elapsed since rhegmatogenous retinal detachment.

Table 3 Idiopathic macular pucker

No	EMB11 (pan macrophage)	27E10 (inflammatory phase macrophage)	RM3/1 (healing phase macrophage)	Duration (months)
1	–	–	–	8
2	(+)	–	(+)	15
3	–	–	–	16
4	–	–	–	20
5	–	–	–	21
6	–	–	–	28

Staining intensity: + = Few macrophages scattered over the membrane or cluster of macrophages. (+) = Single macrophage. – = No staining detected.
Duration = months elapsed since first diagnosis.

Table 4 Traumatic proliferative vitreoretinopathy

No	EMB11 (pan macrophage)	27E10 (inflammatory phase macrophage)	RM3/1 (healing phase macrophage)	Medical history	Duration (months)
1	++	+	–	iofb, ppv, lc	4
2	(+)	–	–	iofb, ppv, sili	4
3	+	+	(+)	iofb, ppv, lc	4
4	++	++	–	lc, cryo	4
5	+	+	–	ppv, sili	4
6	–	–	–	iofb, ppv, sili	5
7	++	+	+	iofb, ppv, sili, cryo	5
8	–	–	–	cryo,lc	5
9	+	+	+	iofb, ppv, sili, lc	6
10	++	++	+	ppv, cryo	6
11	++	++	+	iofb, ppv, sili, lc	8
12	+	(+)	(+)	ppv, cryo, sili, lc	9
13	–	–	–	ppv, sili	12
14	–	–	–	ppv, sili, lc	12
15	–	–	–	iofb, gas	14
16	+	–	(+)	iofb, ppv, sili	24
17	–	–	–	ppv, sili, lc	29
18	–	–	–	iofb, ppv	72
19	(+)	–	–	iofb, cryo, ppv	72

Staining intensity: ++ = Large number of macrophages detected throughout the membrane. + = Few macrophages scattered over the membrane or cluster of macrophages. (+) = Single macrophage. – = No staining detected.
Duration = months elapsed since trauma or last surgical intervention.
Medical history: iofb = intraocular foreign body; cryo = cryocoagulation; lc = laser coagulation; ppv = pars plana vitrectomy; gas = fluid gas exchange; sili = silicone oil.

Discussion

Multiple studies[1-9] have suggested a major role of macrophages as the primary driving force in the pathogenesis of PVR. Macrophages are not only able to stimulate chemotaxis and induce fibroplasia through secretion of fibronectin and leucotrienes, but they also influence cellular proliferation through the synthesis of platelet derived growth factor (PDGF), fibroblast growth factor (FGF), interleukin 1 (IL-1) and transforming growth factor beta (TGF-β).[10 11] However, the mononuclear phagocyte system comprises a wide variety of different cells. Even culture procedures may exert influence on the functional state of macrophages.[12] Therefore the use of monoclonal antibodies on cryostat sections as performed in our series of experiments may provide knowledge of the actual functional state of macrophages at the time of surgical intervention.

In all membranes, where macrophages were detected, a positive labelling with the EBM11 antibody was observed. This is not surprising, since EMB11 has been shown to be broadly reactive with most cells of the mononuclear phagocyte system.[13] However, several membranes with positive labelling of EMB11 did not show reactivity with either 27E10 or RM3/1. Although the function of RM3/1 positive macrophages has yet to be determined, the tissue distribution of these macrophages in an experimental model of gingivitis was associated with the healing phase of tissue following inflammation and the down regulatory phase of immune response.[14] On the contrary large numbers of 27E10 positive macrophages were detected in the acute inflammatory state of the disease.[15 16] Our results confirm previous findings, that macrophage involvement indicated by positive staining of EBM11 is more pronounced in traumatic PVR than in PVR following rhegmatogenous retinal

detachment.[2] In traumatic PVR, 27E10 inflammatory macrophages were restricted to the first 9 months after trauma or vitrectomy. This is in accordance with the fact that macrophages have been shown to play the initiating role of the inflammatory process in proliferative vitreoretinal disorders because of their stimulatory secretion products.[17] In traumatic PVR not only was the number of EBM11 positive macrophages much higher than in PVR following rhegmatogenous retinal detachment and in idiopathic macular pucker, but also the absence of 27E10 positive macrophages indicates a lower degree of inflammatory activity in these disease entities. The colocalisation of 27E10 and RM3/1 positive macrophages is another clue to the heterogenicity of intravitreal proliferation with inflammatory processes and healing processes taking place at the same time.

The source of macrophages in PVR and PDR, whether blood derived or stemming from resting intraocular cells of the mononuclear phagocyte system like retinal pigment epithelium or microglia,[9] has yet to be clarified. The positive finding of macrophages in most of the diabetic membranes may be yet another clue to the idea of blood monocytes differentiating into resting macrophages since the disturbance of the blood-ocular barrier and intraocular bleeding in diabetic retinopathy allows blood monocytes to enter the vitreous cavity in a great number.

Our finding, that macrophages of the acute inflammatory type (27E10) are more abundant in membranes from patients with a more acute and severe progression of proliferative disease, leads to the conclusion that in the case of new pharmacological concepts steroids may be of crucial importance as an adjunctive treatment to surgical intervention because of their suppression of macrophage synthesis and release of inflammatory cytokines and lysosomal enzymes.[18-21] In PVR, experimental studies have demonstrated the reduction of traction retinal detachment in eyes following intraocular injection of steroids[22] or pretreatment with steroids.[23] In another study, a combination therapy of steroids with the cytotoxic drug daunorubicin significantly lowered the rate of neovascularisation compared with treatment with daunorubicin alone.[24] Glucocorticoids have also been shown to induce appearance of the RM3/1 subtype in macrophages.[25] Since this subtype is connected with the healing phase of inflammation and possibly reduced production of prostaglandins in these macrophages,[25] we agree with other authors[26] and suggest that an early pharmacological intervention with corticosteroids may be helpful in inhibiting the initial macrophage activation to prevent subsequent cellular migration and proliferation in PVR and PDR.

This study was supported by the Retinovit Foundation and Deutsche Forschungsgemeinschaft (Wi 880/3-2).

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