CXCR4 expression in vitreoretinal membranes

L Cabay, F Willermain, C Bruyns, JM Verdebout, Y Witta, J Baffi, T Velu, J Libert, L Caspers-Velu, A Maho, L Lespagnard

Background/aim: Proliferative vitreoretinopathy (PVR) and macular pucker (MP) vitreoretinal membranes are caused by abnormal cell migration. By their role in chemotaxis, chemokine receptors represent good candidates to sustain this process. The authors thus investigated the expression of one of them, CXCR4, in these pathologies.

Methods: Three PVR and four MP membranes were surgically removed and processed for immunochemical studies with antibodies for CXCR4, cytokeratins or smooth muscle actin.

Results: CXCR4 expression was found in all membranes. There was no relation between severity of PVR or MP and presence of CXCR4. In addition, there was no difference in CXCR4 expression between MP and PVR.

Conclusion: CXCR4 is expressed in PVR and MP. Further experiments are needed to test if CXCR4 and other chemokine receptors are implicated in vitreoretinal membrane formation.

Materials and Methods

A total of seven VRM were analysed: four MP and three PVR. All MP were idiopathic and all PVR were secondary to retinal detachment surgery. In all cases, surgery consisted of a pars plana vitrectomy with peeling of the membrane from the retina and extraction of the tissue from the vitreous. All specimens were then fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin wax. Immunohistochemical studies were performed on 4 µm thick paraffin sections using a Ventana Nexes Staining System (Ventana Medical Systems, Tucson, AZ, USA), and a DAB detection kit provided by the manufacturer (Ventana). Antibodies directed against the following antigens were used: CXCR4 (clone 12G5, dilution 1:50, Pharmingen, BD Bioscience, Heidelberg, Germany), cytokeratins AE1/AE3 (Dako, A/S, Glostrup, DK), smooth muscle actin SMA (clone 1A4, prediluted), as well as an isotypic control (clone G155–178, Pharmingen, dilution 1:50). Briefly, after 30 minutes of incubation at 37°C with primary antibody, sections were incubated for 10 minutes at 37°C with secondary biotinylated antibody, then with avidin–peroxidase for the same time; 3′,3-diaminobenzidine (DAB) was used as chromogen. Slides were counterstained with haematoxylin and eosin, dehydrated, and mounted before observation under an optical microscope. Positive cells were identified by a brown stain. Slides were carefully analysed by two independent pathologists.

Results

CXCR4 expression was found in all VRM (Fig 1 and Table 1). There was no relation between the severity of PVR or MP and the presence of CXCR4. In addition, there was no difference in CXCR4 expression observed between MP and PVR (Table 1). Results from cell characterisations are summarised in Table 1. Haematoxylin and eosin sections revealed that PVR specimens were heterogeneous with the presence of fusiform cells, pigmented cells, and inflammatory infiltrates. This cell diversity was reflected by immunostaining: one PVR specimen was positive for SMA, one was positive for cytokeratins AE1/AE3, and one was positive for both. In contrast, MP were homogeneous, composed of elongated fusiform cells and few pigmented cells. Accordingly, all MP specimens were positive for SMA and negative for cytokeratins AE1/AE3. CXCR4 positivity was observed in all cell types.

Discussion

One of the most important steps of VRM formation is abnormal cell migration. Chemokines and chemokine receptors are specialised in cell migration control. Chemokine receptors expression allows cells to migrate in response to a gradient of chemokine ligands.

If the implication of chemokines in VRM has been highlighted by others, to our knowledge none has yet reported on the potential role of chemokine receptors. Here we show that CXCR4 is expressed in pathological specimens of VRM (PVR and MP).
CXCR4 and its ligand SDF-1 differ from other chemokine-ligand pairs. Firstly, CXCR4 is one of the very few chemokine receptors for which only a single ligand has been identified so far. Secondly, CXCR4 and SDF-1 are expressed constitutively in a wide range of tissues including brain, thymus, lymph nodes, spleen, stomach, kidney, and gut. A functional in vitro expression of CXCR4 by RPE cells was also recently demonstrated by Crane et al. They indeed showed that RPE cells expressed both CXCR4 and SDF-1, and that in vitro SDF-1 stimulation resulted in RPE cell migration. Accordingly, we also observed that CXCR4 is expressed in enucleated eyes, on RPE cells (data not shown).

Theoretically, thus, if SDF-1 is produced in vivo, RPE cells should migrate in response to its gradient, as should glial cells, another important cell type in VRM. We did not look for SDF-1 secretion in the vitreous of our patients. But a similar role has been attributed to the CXCR4-SDF-1 pair in the resolution phase of wound healing, a pluricellular process which in many ways mimics VRM formation.

In conclusion, we showed that CXCR4 is expressed in VRM, but further experiments are needed to define the role of CXCR4 and other chemokine receptors in VRM formation.

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