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The activation of IL-8 receptors in cultured guinea pig Müller glial cells is modified by signals from retinal pigment epithelium☆

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Abstract

Interleukin 8 (IL-8, CXCL8) is a pro-inflammatory chemokine which attracts neutrophils to sites of inflammation via an activation of the G-protein-coupled receptors, CXCR1 and CXCR2. However, both IL-8 and IL-8 receptors are widely expressed in various tissues and cell types, and have been suggested to be involved in other functions such as angiogenesis, tumor growth, or brain pathology. We examined the expression of IL-8 and IL-8 receptors in highly enriched primary cultures of guinea pig Müller glial cells. Immunoreactivity for CXCL8, CXCR1 and CXCR2 was observed in all cultured Müller cells. The expression of CXCL8 was confirmed by PCR, and the secretion of the CXCL8 protein from Müller cells was revealed by ELISA. Western blots showed prominent bands at ~40 kDa by using antibodies specific for human CXCR1 and CXCR2, and the expression of a putative CXCR2 receptor in Müller cells was confirmed by PCR. Furthermore, cultured Müller cells responded to application of recombinant human IL-8 with an increase of the cytosolic Ca^{2+} concentration. If supernatants of cultured human retinal pigment epithelium (RPE) cells were applied to the Müller cell cultures, no obvious changes were observed in the CXCL8, CXCR1 and CXCR2 expression but (i) Müller cell proliferation was stimulated, and (ii) there was an increased number of CXCL8-responsive Müller cells and the amplitudes of the evoked calcium responses were enhanced. It is concluded that Müller glial cells may participate in the inflammatory response(s) of the retina during ocular diseases, and that this contribution may be modified by interactions with RPE cells.

Keywords: Müller cell; Glia; CXCL8; CXCR1; CXCR2; Chemokine; Guinea pig

1. Introduction

Interleukin 8 (IL-8, CXCL8), a pro-inflammatory chemokine, belongs to a family of structurally related peptides of 8–10 kDa, and was originally identified as a neutrophil-activating protein (Baggiolini and Clark-Lewis, 1992). The amino acid sequences of IL-8 are known for several species (e.g. human, rabbit, and guinea pig) and show strong sequence similarities between these species (Beaubien et al., 1990; Walz et al., 1987; Yoshimura and Johnson, 1993). Many different cell types, such as monocytes, macrophages, endothelial cells, epithelial cells, and fibroblasts, are able to secrete IL-8 in response to inflammatory stimuli (Atta et al., 1999; Baggiolini and Clark-Lewis, 1992). Additionally in the CNS, IL-8 is expressed by microglia cells (Atanassov et al., 1995; Ehrlich et al., 1998; Hua and Lee, 2000; Xia and Hyman, 1999) and/or astrocytes (Aloisi et al., 1992, 1995; Lacy et al., 1995; Saas et al., 1999; Xia and Hyman, 1999)

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under physiological or pathological conditions. IL-8 was detected in astrocytomas and glioblastomas as well as in cell lines derived from malignant astrocytes (Atanassov et al., 1995; Desbaillets et al., 1997; Nitta et al., 1992; Oh et al., 1999). Several functions of IL-8 were suggested for different cell types and in different tissues, including neutrophil recruitment, cell adhesion, homing of neutrophils and lymphocytes, tumor growth, angiogenesis, neuronal protection and brain development (Hesselgesser and Horuk, 1999; Mackay, 2001; Mennicken et al., 1999; Rossi and Zlotnik, 2000). In neutrophils, IL-8 induces three main responses, (i) shape changes and directional migration, (ii) exocytosis of storage proteins and (iii) the respiratory burst, which is characteristic for stimulated phagocytes (Baggiolini and Clark-Lewis, 1992).

The action of IL-8 in neutrophils is mediated by the activation of two distinct high affinity IL-8 receptors, designated as CXCR1 and CXCR2 (Murphy et al., 2000). Both receptors belong to a subfamily of heptahelical transmembrane receptors, and are coupled to heterotrimeric G-proteins (Atta et al., 1999; Baggiolini and Clark-Lewis, 1992). The activation of the IL-8 receptors triggers the formation of distinct second messengers, which activate several signaling pathways via activation of protein kinases and phospholipases (Atta et al., 1999). One of the main signal transduction pathways is the activation of the phospholipase C, which induces the generation of diacylglycerol and inositol 1,4,5 triphosphate, and causes a transient mobilization of free Ca^{2+} from internal stores.

In addition to their localization in neutrophils and monocytes/macrophages, CXCR1 and CXCR2 have been detected in stimulated eosinophils, basophils, T-lymphocytes and dendritic cells (Murphy et al., 2000). CXCR2, but not CXCR1, has been identified on subsets of neurons in the human CNS by immunohistochemical methods (Horuk et al., 1997). Rat septal cholinergic neurons express CXCR1 and CXCR2 (Puma et al., 2001). The expression of CXCR2 in cultured neonatal astrocytes and in the adult human brain was also detected (Dorf et al., 2000). Recently, the expression of CXCR1 and CXCR2 on cultured human microglial cells and astrocytes was described (Flynn et al., 2003). The expression of CXC receptors in different cell types suggests, in addition to their well-established role in the immune system, their involvement in brain development, various neurological disorders, neuroinflammatory processes, and neuroimmune diseases (Biber et al., 2002; Halks-Miller et al., 1997; Hesselgesser and Horuk, 1999; Mennicken et al., 1999; Xia and Hyman, 1999).

Müller cells are the dominant macroglial cells in the neuronal retina (and even the only macroglial cell type in avascular retinae such as that of the guinea pig). Müller (radial glial) cells span the entire thickness of the retina, and contact and ensheath virtually every type of neuronal somata and cell processes. This morphological relationship is reflected by a multitude of interactions between neurons and Müller cells in normal and pathologically altered retinae (Bringmann and Reichenbach, 2001; Newman and Reichenbach, 1996). The vast majority of (if not all) pathological retinal alterations are accompanied by a reactive gliosis involving distinct changes of Müller cell properties and functions (Bringmann and Reichenbach, 2001; Reichelt et al., 1997). For instance, retinal detachment (RD) and proliferative vitreoretinopathy (PVR) are major causes of severe visual loss. In both cases, Müller cells become hypertrophic and express the intermediate filament, glial fibrillary acidic protein (GFAP), as an indicator of a reactive gliosis (Francke et al., 2001a; Okada et al., 1990). Furthermore, they transdifferentiate, migrate, proliferate, and form (together with other cell types such as retinal pigment epithelial cells and leukocytes) deleterious cellular membranes (Bringmann et al., 2000; Charteris, 1995; Fisher et al., 1991; Nork et al., 1986).

Increased levels of IL-8 were detected in the vitreous of patients with PVR or proliferative diabetic retinopathy (Aksunger et al., 1997; El Ghrably et al., 2001; Elner et al., 1998). The exact intraocular cellular source(s) of this and other chemoattractants to recruit neutrophils, monocytes and/or lymphocytes are largely unknown. Recently, a guinea pig equivalent of the human CXCR2 receptor was identified in non-neuronal tissues, but the existence of the CXCR1 equivalent in guinea pigs is still uncertain (Catusse et al., 2003). Here we show that cultured Müller glial cells from the guinea pig retina (i) produce CXCL8 and (ii) express (two subtypes of) functional IL-8 receptors which (iii) induce intracellular Ca^{2+} rises upon stimulation and whose efficacy (iv) is modulated by soluble factors from retinal pigment epithelial cells.

2. Material and methods

2.1. Guinea pig Müller cell cultures

Animal care and handling were performed in accordance with applicable German laws and with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Primary cultures of Müller glial cells were obtained from retinae of adult guinea pigs weighing 250 g to 300 g. Animals were killed by an overdose of urethane (2 g/kg, 5 ml, i.p.) before decapitation and enucleation of the eyes. The excised retinae were dispersed in calcium and magnesium-free phosphate buffer (PBS) supplemented with nagarse (1 mg/ml) for 30 min at 37 °C. After washing in phosphate buffer, containing DNase I (200 units/ml), the dissociated cells were seeded on uncoated coverslips (diameter 15 mm; Glaswarenfabrik Hecht, Sontheim/Rhön, Germany). Cells were cultured in minimal essential medium (MEM, M4642) supplemented with 10% fetal calf serum (FCS) at 37 °C in 95/5% air/CO_2. Just before achieving confluency after 6–8 days, some Müller cell cultures were stimulated three times (every second day) with supernatants from separate cultures of human retinal pigment epithelium cells (RPE). Unfixed