Available online at www.sciencedirect.com
SCIENCE DIRECT
Experimental Eye Research 80 (2005) 369–378
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Quantitative enumeration of vascular smooth muscle cells and endothelial cells derived from bone marrow precursors in experimental choroidal neovascularization

Diego G. Espinosa-Heidmann[a,1,*], Maria A. Reinoso[a,1], Yolanda Pina[a], Karl G. Csaky[b], Alejandro Caicedo[a], Scott W. Cousins[a]

[a]*Department of Ophthalmology, Bascom Palmer Eye Institute, William L. McKnight Vision Research Center, The University of Miami School of Medicine, 1638 N.W. 10th Avenue, Miami, FL 33136, USA*
[b]*The National Eye Institute, National Institute of Health, Bethesda, MD, USA*

Received 16 July 2004; accepted in revised form 5 October 2004

Abstract

Choroidal neovascularization (CNV) is characterized by the subretinal invasion of a pathologic new vessel complex from the choriocapillaris. Although CNV is traditionally considered to consist of endothelial cells, the cellular population of CNV is likely more complex in nature, comprising several different cell types. In addition, recent studies suggest that the CNV cell population has a dual origin (circulating versus resident populations). In this study we sought to determine the contribution and origin of different cell types in experimental CNV. Laser-induced CNV was performed on chimeric mice generated by reconstituting C57BL/6 mice with bone marrow from green fluorescent protein (GFP)-transgenic mice. In these mice, bone marrow-derived cells are GFP-labeled. Immunofluorescence staining was used to examine both flatmount preparations of the choroid and cross sections of the posterior pole for macrophages, endothelial cells, vascular smooth muscle cells, retinal pigment epithelial (RPE) cells, lymphocytes, or neutrophils at day 3, 7, 14 and 28 post-laser ($n=5$ per group). Cell types present in CNV included macrophages (20% of the cells in CNV), endothelial cells (25%), vascular smooth muscle cells (11%), RPE cells (12%) and non-labeled cells (32%). The macrophage population was mostly derived from circulating monocytes at all timepoints studied (70% were GFP labeled), while endothelial and vascular smooth muscle cells were partly bone marrow derived (50–60% were GFP labeled), and RPE cells appeared to be entirely derived from preexisting tissue resident cells. These results demonstrate that bone marrow-derived progenitor cells contribute significantly to the vascular and inflammatory components of CNV. Knowledge of the cellular composition and origin might help understand the pathogenic mechanisms controlling CNV severity as well as indicate potential targets for therapeutic intervention.

Keywords: age-related macular degeneration (AMD); choroidal neovascularization (CNV); macrophages; bone marrow-derived vascular progenitor cells; RPE cells; laser photocoagulation; animal model; cellular composition

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly population (Evans, 2001). AMD is a multifactorial disease related to age, systemic health, genetic and environmental risk factors (Evans, 2001). Inflammatory and immune mechanisms have recently been hypothesized to play a role in the pathogenesis of the disease (Penfold et al., 1985, 2001). Choroidal neovascularization (CNV) is the major complication

* Corresponding author. Dr Diego G. Espinosa-Heidmann, Department of Ophthalmology, Bascom Palmer Eye Institute, William L. McKnight Vision Research Center, The University of Miami School of Medicine, 1638 N.W. 10th Avenue, Miami, FL 33136, USA.
E-mail address: despinosa@med.miami.edu (D.G. Espinosa-Heidmann).
[1] These authors contributed equally to this work and therefore should be considered equivalent senior authors.

0014-4835/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2004.10.005

associated with vision loss in AMD (Young, 1987; Starr et al., 1998). The morphology and cellular composition of CNV is much more complex than the traditional model of CNV that conceptualizes this lesion as endothelial lined capillary tubes. Histopathology has demonstrated a wide degree of variable cellularity and fibrosis among different lesions (Grossniklaus et al., 2002). Thus, CNV are composed of many different cell types in addition to endothelial cells (Lopez et al., 1991; Seregard et al., 1994). These include RPE cells, myofibroblasts, vascular smooth muscle cells, CD 34 progenitor cells as well as inflammatory cells such as macrophages (Lopez et al., 1991, 1996; Grossniklaus et al., 1992; Thomas et al., 1993; Csaky et al., 2004a,b). However, no systematic detailed analysis has been conducted on the origin of the different cell populations and their contribution to human or experimental CNV.

Inflammatory cells, specifically blood-derived macrophages, are the most obvious nonocular cell type that might be recruited to CNV. Macrophages can either be tissue-resident macrophages (which appear to be recruited early in ocular development and are long lived within the choroid) or blood-derived macrophages, which form part of the leukocyte population that is responsible for the innate immune response (Espinosa-Heidmann et al., 2003a,b; McMenamin, 1997, 1999). Their importance has been recently confirmed in both human and experimental CNV (Grossniklaus et al., 2000, 2002; Espinosa-Heidmann et al., 2003a,b; Sakurai et al., 2003).

The traditional paradigm of angiogenesis assumes that vascular cells in CNV are derived only from preexisting resident cells from the adjacent normal vascular bed. However, recruitment of vascular cells from a circulating population of cells has become another potential important source of cells forming pathological or physiological neovascularization, including CNV (Asahara et al., 1999a). Our group as well as other laboratories has demonstrated that circulating bone marrow-derived vascular precursor cells also contribute significantly to CNV composition (Espinosa-Heidmann et al., 2003a; Sengupta et al., 2003).

Circulating precursor cells have been noted to exist for a wide range of populations, including epithelial cells (hepatocytes, pneumocytes), myocardiocytes, endothelial cells, VSMC, glial cells and even neurons (Asahara et al., 1997, 1999b; Isner and Asahara, 1999; Cornacchia et al., 2001; Religa et al., 2002; LaBarge and Blau, 2002; Otto, 2002; Bailey and Fleming, 2003; Kajstura et al., 2004; Black et al., 2004; Picard-Riera et al., 2004). Recent studies have shown that vascular cells within CNV are derived from circulating precursors, (Espinosa-Heidmann et al., 2003a; Sengupta et al., 2003) but their relative contribution to the different cell types has not been studied. Surprisingly, the published studies have focused mostly on the recruitment of endothelial progenitor cells to sites of neovascularization, and ignored the contribution of marrow precursors to other cell types within neovascular tissues, including CNV (Csaky et al., 2004a,b).

In this study, we examined the contribution of different cell types to CNV using immunohistochemistry of mouse eyes with experimental CNV. We used markers for macrophages, endothelial cells, vascular smooth muscle cells, RPE cells, lymphocytes, and neutrophils. We further sought to determine the relative contribution of circulating precursor cells and monocytes to each particular cell population within developing CNV at different time points. Not surprisingly, we found that blood-derived macrophages were the predominant form of monocyte at all time points during CNV formation. We also observed that a significant frequency of endothelial and vascular smooth muscle cells were bone marrow-derived, whereas all RPE cells appeared to be entirely derived from preexisting tissue resident cells.

2. Materials and methods

2.1. Mice

Mice used in this study were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A total of 20 female C57BL/6 mice 9–11 months old were purchased from The Jackson Laboratory (Bar Harbor, ME) and used as recipient mice for bone marrow transplantation. Bone marrow was obtained from seven adult C57BL/6 female mice transgenic for the chicken β-actin promoter-GFP and cytomegalovirus enhancer (The Jackson Laboratory).

2.2. Bone marrow transplant

Briefly, donor mice were anesthetized with an intramuscular administration of ketamine hydrochloride ($42{\cdot}8\ mg\ kg^{-1}$), xylazine ($8{\cdot}5\ mg\ kg^{-1}$) and acepromazine ($1{\cdot}4\ mg\ kg^{-1}$). Tibias and femurs were dissected and bone marrow extracted by slowly flushing medium inside the diaphyseal channel with a 27-gauge needle. Bone marrow was homogenized, filtered, centrifuged, and resuspended as previously described (Espinosa-Heidmann et al., 2003a). Recipient mice were lethally irradiated (950 cGy) and given 10^7 nonpurified bone marrow cells intravenously. Blood components were allowed to reconstitute for 1 month.

2.3. CNV induction

One month after transplantation, diode red laser was used to create four choroidal thermal burns around the optic nerve to induce experimental CNV in both eyes. Four weeks after application of laser, mice were sacrificed as previously described at different time points (3 days, 1 week, 2 weeks, and 4 weeks; $n=5$/group) for immunohistochemistry