ELSEVIER

Quantitative Analysis of Major Histocompatibility Complex Class II-Positive Cells in Posterior Segment of Royal College of Surgeons Rat Eyes

Keiko Akaishi, Sei-ichi Ishiguro, Yusuf Kemal Durlu and Makoto Tamai

Department of Ophthalmology, Tohoku University School of Medicine, Sendai 980-77, Japan

Abstract: Potential antigen-presenting cells in the posterior segment of Royal College of Surgeons (RCS) rat eyes were analyzed quantitatively. Light microscopic immunohistochemistry was performed at postnatal days (P) 10, 20, 28, 42, 63, and 140 in the eyes of RCS rats and their congenic counterparts. Immunohistochemical studies were carried out using monoclonal antibodies against major histocompatibility complex (MHC) class II antigen (OX6), a cytoplasmic antigen in bone marrow-derived macrophages (ED1), a membrane antigen on resident tissue macrophages (ED2), and a microglia/macrophage marker (OX42). Some sections were stained by a double-labeling method using these antibodies. No MHC class II-positive cells were seen in dystrophic RCS rat retinas at P10. They were found, however, in the outer nuclear layer and debris of outer segments at P20. From P20 to P42 the number of cells increased, then decreased until P140. Congenic controls, however, showed no MHC class II-positive cells in the retina. Cells double-labeled with OX6 and ED1 were present in the outer nuclear layer at P42, but no OX6 or OX42 double-labeled cells were detected. Also, no ED2-positive cells were detected. Our results suggest that MHC class II-positive cells may play some role in retinal dystrophy. **Jpn J Ophthalmol 1998;42:357–362** © 1998 Japanese Ophthalmological Society

Key Words: Macrophage, MHC class II, microglia, RCS rat, retina.

Introduction

The Royal College of Surgeons (RCS) rat has an inherited retinal dystrophy that produces a progressive loss of photoreceptor cells. Photoreceptor cell dystrophy begins in the third postnatal week at late stages of retinal development, and most of the cells that compose the outer nuclear layer disappear by the third month of life. A retinal pigment epithelial (RPE) defect has been implicated in the retinal degeneration of the RCS rat. From postnatal day (P)20, increased pyknosis in the outer nuclear layer is the first morphological sign of retinal dystrophy. Then, within 1 month, a rapid and complete photoreceptor cell loss occurs at the neural retina in this animal model.[1–4]

Major histocompatibility complex (MHC) is a genetic region in all mammals where immunological events, such as immune responses and rejection following transplantation, are regulated.[5,6] The role of MHC is to initiate signaling between lymphocytes and foreign epitopes. MHC class II-positive cells could not be found in normal rat retina, but the positive cells were abundant in the iris and ciliary body of the rat, mouse, and man.[7–9] Human RPE cells have expressed MHC class II antigens in response to in vitro stimulation by the lymphokine, interferon-γ.[10] It has also been reported that the antigen is induced by the inoculation of interferon-γ[11] and is found in experimental autoimmune uveoretinitis[12] in rat RPE cells. Autoimmune response was detected in many degenerative ocular disorders, but it is not known if they play a contributory pathogenic role.[13] Optic nerve degeneration induces the expression of MHC antigens in the rat visual system.[14] Microglial cells, which are the resident macrophages in the central nervous system, may be important in antigen presentation in neural tissue, but direct evidence of their role has not yet been found.

Received: July 16, 1997

Address correspondence and reprint requests to: Keiko AKAISHI, MD, Department of Ophthalmology, Tohoku University School of Medicine, 1-1 Seiryo, Aoba-Ku, Sendai, 980-77 Miyagi Japan

Published by Elsevier Science Inc.

0021-5155/98/$19.00
PII S0021-5155(98)00035-5

Jpn J Ophthalmol
Vol 42: 357–362, 1998

To determine whether the MHC class II-positive cells in RCS rat retinas are related to the progression of photoreceptor degeneration, we counted MHC class II-positive cells using monoclonal antibodies against MHC class II antigen (OX6). In addition, we used monoclonal antibodies against a cytoplasmic antigen in bone marrow-derived macrophages (ED1), a membrane antigen on resident tissue macrophages (ED2), and the microglia/macrophage marker (OX42) to identify MHC class II-positive cells.

Materials and Methods

Animals

RCS dystrophic rats (rdy/rdy) at P10, 20, 28, 42, 63, and 140 and their age-matched congenic counterparts (rdy+/rdy+) (n = 3) were kept under the conditions of a 12-hour light and 12-hour dark cycle. The experiments were conducted according to the guidelines described in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Immunohistochemistry

Frozen sections (10 μm) from the posterior pole of the eyeball were placed on poly-L-lysine-coated slides. Sections were air-dried and fixed with cold acetone. The sections were rehydrated with phosphate-buffer saline (PBS) and incubated with primary antibodies in 0.05% Tween-20-PBS (Tween-PBS) containing 5% skim milk for 3 hours in a humid chamber. The sections were washed five times in Tween-PBS. Monoclonal antibodies used were OX6 (recognizing rat MHC class II antigen), OX42 (recognizing rat microglia, monocyte), ED1 (recognizing a cytoplasmic antigen in rat monocytes, macrophages, and 90% of dendritic cells), and ED2 (recognizing a membrane antigen resident tissue macrophage). OX6 and OX42 were obtained from Pharmingen (San Diego, CA, USA). ED1 and ED2 were obtained from Serotec (Oxford, UK). An avidin-biotinylated peroxidase complex (ABC) method using biotinylated antibody, streptavidin-biotin-peroxidase complex (DAKO, Glostrup, Denmark), and an enzyme-labeled antibody method (indirect) with 3,3′-diaminobenzidine tetrahydrocholoride as the substrate were used in this study. All procedures were carried out at room temperature. Endogenous peroxidase activity was eliminated by incubating the sections in PBS containing 0.3% H_2O_2 and 0.1% sodium azide for 20 minutes. Sections were counterstained with methylene green and mounted. Some

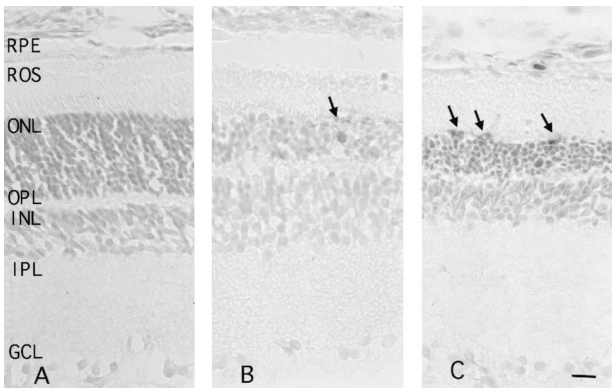

Figure 1. Immunohistochemical findings using monoclonal antibody against major histochemically complex (MHC) class II antigen (OX6) in Royal College of Surgeons (RCS) dystrophic rats and control congenic counterparts. (**A**) No MHC class II-positive cells existed in retinas of congenic control of RCS rat. RPE: retinal pigment epithelium, ROS: photoreceptor rod outer segments, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, and GCL: ganglion cell layer. (**B**) MHC class II-positive cells (arrow) existed in outer nuclear layer of RCS dystrophic rats at postnatal day (P) 25. (**C**) MHC class II-positive cells (arrows) were found in outer nuclear layer together with debris of rod outer segments of RCS dystrophic rats at P42. Most positive cells appeared dendritic. Bar = 25 μm.

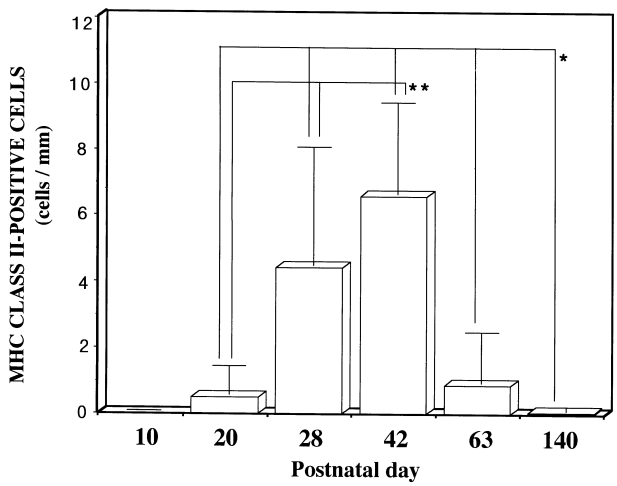

Figure 2. Quantitative analysis of major histochemically complex (MHC) class II-positive cells in Royal College of Surgeons (RCS) dystrophic rats. Positive cells in retina were quantified as cells/mm (mean ± SD). MHC class II-positive cells numbered 0.5 ± 0.8 at P20, 4.43 ± 3.55 at P28, 6.57 ± 2.79 at P42, 0.86 ± 1.56 at P63 and 0.04 ± 0.12 at P140. There were fewer MHC class II-positive cells at P140 than at P20, 28, 42, or 63 (*$P < 0.01$ Mann-Whitney U test). MHC class II-positive cells at P28 and P42 were more abundant than at P20 (**$P < 0.01$ Mann-Whitney U test).

sections were double-stained using OX6 and ED1 antibodies. OX6 and ED1 antibodies were detected with peroxidase-labeled second antibody and alkaline phosphatase-labeled second antibody, respectively. Color development for alkaline phosphatase was carried out with fast red (DAKO). Endogenous alkaline phosphatase activity was eliminated by 2.5 mmol/L levamisole. In this case, no counterstaining was carried out, and the sections were mounted with glycerol.

Quantitative Analysis

Immunostained cells in the posterior retinas were counted at P10, 20, 28, 42, 63, and 140 under a light microscope. Sections were taken at 30-μm intervals. Three rats were examined on each postnatal day. The mean number of cells per millimeter of retinal length was calculated, and the mean ± SD was determined for each group. Data were analyzed using the Mann-Whitney *U* test.

Results

As shown in Figure 1, most positive cells had a dendritic appearance and were present in the outer nuclear layer in the RCS dystrophic rats. Some of the immunoreactive cells were also found in the debris of rod outer segments. No MHC class II-positive cells were seen at P10, but immunoreactive cells were found at P20. A gradual increase in MHC class II-positive cells occurred from P20 to P42, then they decreased at P63. At P140, very few MHC class II-positive cells were found. Congenic control retinas at P10, 20, 28, 42, 63, and 140 showed no MHC class II-positive cells in the retina. Quantitative data on MHC class II-positive cells from P10 to P140 are shown in Figure 2.

Identification of MHC class II-positive cells was carried out comparing the P42 retinas of RCS dystrophic rats and their congenic counterparts. The ED1-positive cells were seen at the outer nuclear layer of RCS dystrophic rat retinas (Figure 3). We could find no ED1-positive cells in the congenic control retinas. Furthermore, no ED2-positive cells were seen in either the dystrophic or control retinas (Figure 4). OX42-positive cells were found in the inner nuclear layer, inner plexiform layer and ganglion cell layer in the retinas of congenic control RCS rats, and in all layers in the retinas of RCS dystrophic rats (Figure 5).

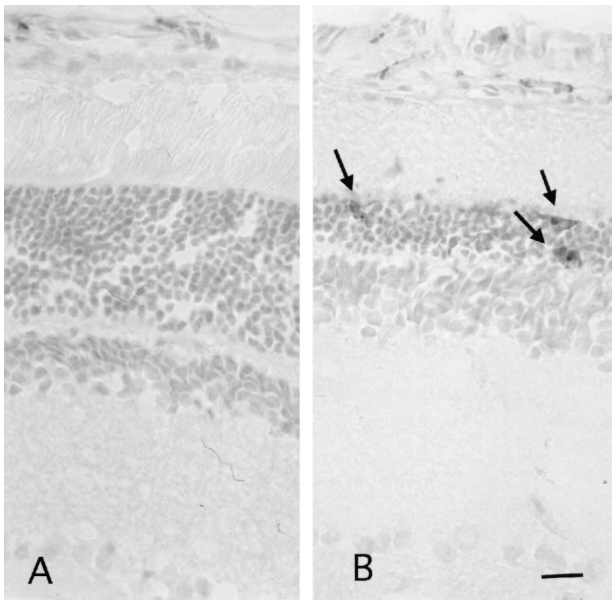

Figure 3. Immunohistochemical findings using monoclonal antibody against cytoplasmic antigen in bone marrow-derived macrophages (ED1) of Royal College of Surgeons (RCS) rats and control congenic counterparts. (**A**) No ED1-positive cells existed in retinas of congenic control RCS rats. (**B**) ED1-positive cells (arrows) existed in outer nuclear layer of RCS dystrophic rat retina at P42. Bar = 25 μm.

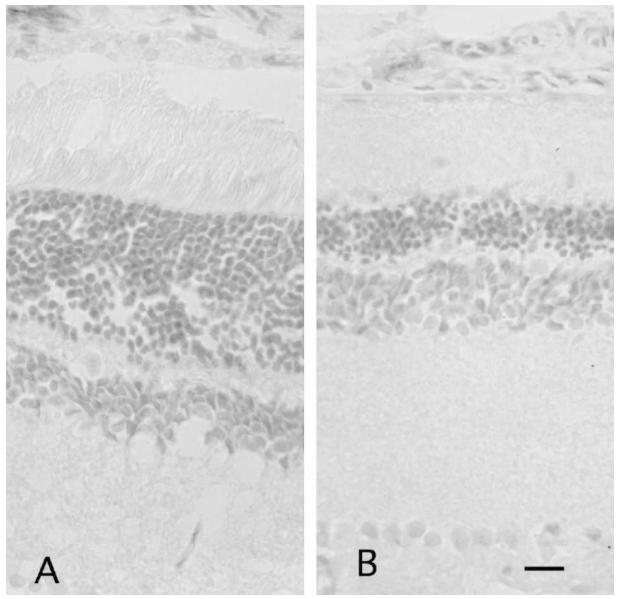

Figure 4. Immunohistochemical findings using monoclonal antibody against membrane antigen on resident tissue macrophages (ED2) of Royal College of Surgeons (RCS) rats and control congenic counterparts. (**A**) No ED2-positive cells existed in retinas of congenic control RCS rats, (**B**) or in retinas of RCS dystrophic rats at P42. Bar = 25 μm.

Jpn J Ophthalmol
Vol 42: 357–362, 1998

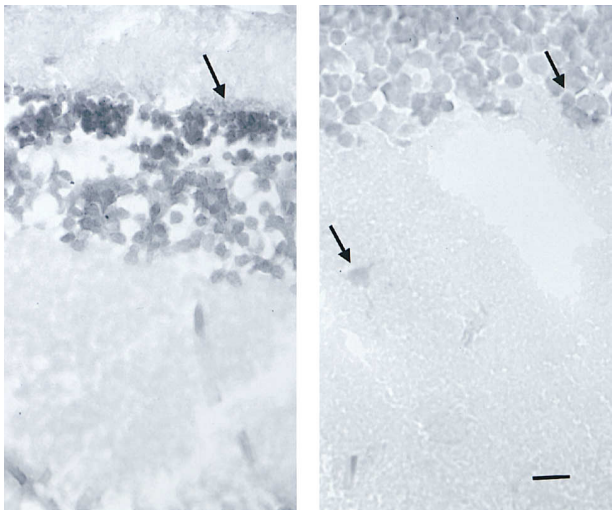

Figure 5. Immunohistochemical findings using monoclonal antibody against microglia/macrophage marker (OX42) of Royal College of Surgeons (RCS) rats and control congenic counterparts. (**A**) OX42-positive cells (arrows) were found in inner nuclear layer, inner plexiform layer and ganglion cell layer in retinas of congenic control counterparts, and (**B**) were found in all layers in retinas of RCS dystrophic rats. Bar = 25 μm.

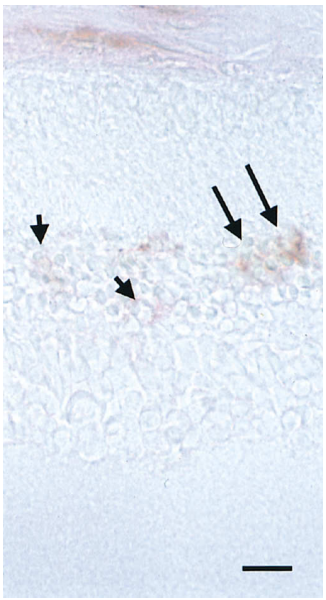

Figure 6. Immunohistochemical findings using monoclonal antibodies against OX6 and ED1 in Royal College of Surgeons (RCS) dystrophic rat. Cells double-labeled (long arrows) by OX6 and ED1 were present at outer nuclear layers in RCS dystrophic rats at P42. Some cells (short arrows) did not express MHC class II antigen. Bar = 25 μm.

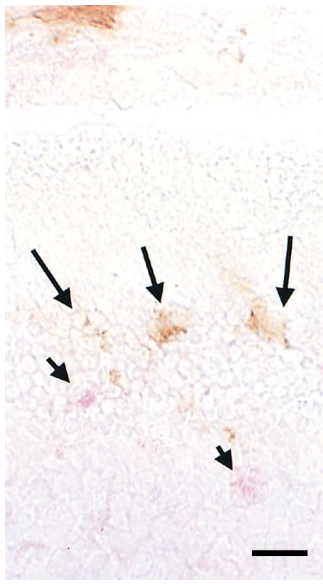

Figure 7. Immunohistochemical findings using monoclonal antibodies against OX6 and OX42 in Royal College of Surgeons (RCS) dystrophic rats. No cells double-labeled by OX6 and OX42 existed in outer nuclear layer in RCS dystrophic rat retinas at P42. OX6-labeled cells (long arrows) have different distributions than OX42-labeled cells (short arrows). Bar = 25 μm.

Cells double-labeled by OX6 and ED1 were present in the outer nuclear layer of RCS dystrophic rats (Figure 6), whereas no cells double-labeled by OX6 and OX42 were detected in the retinas of RCS dystrophic rats (Figure 7). As shown in Figure 6, some bone marrow-derived macrophages were not expressing MHC class II antigen. All immunohistochemical observations in the RCS dystrophic rat are presented in Figure 8.

Discussion

Autoimmune response has been detected in many degenerative ocular disorders.[13] They can aggravate a degenerative process by immunopathological mechanisms, thereby causing the normally protective role of the immune system to turn into a destructive one. Immune responses are initiated by MHC-positive cells, which present antigen to T cells. It is believed that autoimmune mechanisms may play a role in the pathogenesis of retinal dystrophy in the RCS rat.[15] The appearance of MHC class II-positive cells was associated with progressive photoreceptor degeneration. In the present study, we showed that MHC class II-positive cells were first detected at P20, they increased until P42, and then they decreased until P140. Reportedly, debris of outer seg-

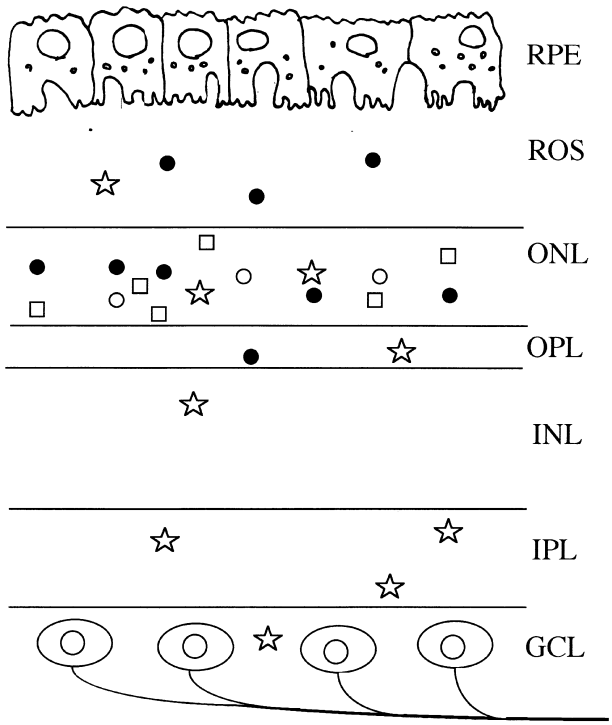

Figure 8. Schematic drawing depicts all immunohistochemical observations in RCS dystrophic rat. ●: OX6-positive cells, □: ED1-positive cells, ○: OX6- and ED1-positive cells, ☆: OX42-positive cells, GCL: ganglion cell layer, IPL: inner plexiform layer, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, ROS: photoreceptor rod outer segments; RPE: retinal pigment epithelium.

ments accumulated at the surface of the pigment epithelium at P20. The outer segment zone became a disorganized debris zone, and many photoreceptor nuclei were degenerating by P32. Then, a rapid and complete photoreceptor cell loss occurred at the neural retina in this animal model within a month. Therefore, MHC class II-positive cells seemed to appear during the early stages of photoreceptor degeneration.[16]

In the retina, microglial cells are believed to be the nonprofessional antigen-presenting cells. OX42-positive cells, which are thought to be microglial cells, existed in the outer retina of the dystrophic RCS rat in the present study. It has been reported that microglial cells invade the outer retina during photoreceptor degeneration in RCS rats. Regarding microglia, our results are consistent with those of Roque et al.[17] Microglial cells are believed to derive from resident tissue and to be attracted to degenerating photoreceptors.[17,18] In the developing mouse retina, macrophages migrate from the vascular supply and phagocytose degenerating neurons.[19] The macrophages subsequently differentiate to become microglia of the retina.

In addition to microglial cells, we found bone marrow-derived macrophages during the early stages of photoreceptor degeneration by using monoclonal antibody ED1. The double-staining method using OX42 and ED1 confirmed that OX42-positive microglial cells and ED1-positive cells are of different origin, since no double-stained cell was present. Interestingly, by using the double-staining method with OX6 and ED1, we found that some ED1-positive cells were presenting MHC class II antigen. It assumed that the character of the surface antigen of macrophages might change during photoreceptor cell degeneration.

Our results suggested that MHC class II-positive cells are mainly bone marrow-derived macrophages, and that they may play some role in retinal dystrophy.

References

1. Bourne MC, Campbell DA, Tansley K. Hereditary degeneration of the rat retina. Br J Ophthalmol 1938;22:613–23.
2. Bok D, Hall MO. The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. J Cell Biol 1971;49:664–82.
3. LaVail MM, Sidman RL, Gerhardt CO. Congenic strains of RCS rats with inherited retinal dystrophy. J Hered 1975; 66:242–4.
4. Goldman AI, O'Brien PJ. Phagocytosis in the retinal pigment epithelium of the RCS rat. Science 1978;201:1023–5.
5. Jiang LQ, Streilein JW. Immunity and immune privilege elicited by autoantigens expressed on syngeneic neonatal neural retina grafts. Curr Eye Res 1992;11:697–709.
6. Subramanian T, Pollack IF, Lund RD. Rejection of mesencephalic retinal xenografts in the rat induced by systemic administration of recombinant interferon-gamma. Exp Neurol 1995;131:157–62.
7. McMenamin PG, Holthouse I, Holt PG. Class II major histocompatibility complex (Ia) antigen-bearing dendritic cells within the iris and ciliary body of the rat eye: distribution, phenotype and relation to retinal microglia. Immunology 1992;77:385–93.
8. McMenamin PG, Crewe J, Morrison S, Holt PG. Immunomorphologic studies of macrophages and MHC class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 1994; 35:3234–50.
9. Forrester JV, McMenamin PG, Holthouse I, Lumsden L, Liversidge J. Localization and characterization of major histocompatibility complex class II-positive cells in the posterior segment of the eye: implications for induction of autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 1994;35:64–77.
10. Liversidge JM, Sewell HF, Forrester JV. Human retinal pigment epithelial cells differentially express MHC class II (HLA, DP, DR and DQ) antigens in response to in vitro stimulation with lymphokine or purified IFN-gamma. Clin Exp Immunol 1988;73:489–94.

Jpn J Ophthalmol
Vol 42: 357–362, 1998

11. Hamel CP, Detrick B, Hooks JJ. Evaluation of Ia expression in rat ocular tissues following inoculation with interferon-gamma. Exp Eye Res 1990;50:173–82.
12. Chan CC, Hooks JJ, Nussenblatt RB, Detrick B. Expression of Ia antigen on retinal pigment epithelium in experimental autoimmune uveoretinitis. Curr Eye Res 1986;5:325–30.
13. Chant SM, Heckenlively J, Meyers-Elliott RH. Autoimmunity in hereditary retinal degeneration. I. Basic studies. Br J Ophthalmol 1985;69:19–24.
14. Rao K, Lund RD. Optic nerve degeneration induces the expression of MHC antigens in the rat visual system. J Comp Neurol 1993;336:613–27.
15. Chant SM, Meyers-Elliott RH. Autoimmunity: a possible factor in the development of retinal degeneration in the RCS rat. Clin Immunol Immunopathol 1982;22:419–27.
16. LaVail MM. Analysis of neurological mutants with inherited retinal degeneration. Friedenwald lecture. Invest Ophthalmol Vis Sci 1981;21:638–57.
17. Roque RS, Imperial CJ, Caldwell RB. Microglial cells invade the outer retina as photoreceptors degenerate in Royal College of Surgeons rats. Invest Ophthalmol Vis Sci 1996;37:196–203.
18. Thanos S. Sick photoreceptors attract activated microglia from the ganglion cell layer: a model to study the inflammatory cascades in rats with inherited retinal dystrophy. Brain Res 1992;588:21–8.
19. Hume DA, Perry VH, Gordon S. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 1983;97:253–57.