Retinal Degeneration under the Effect of Antibodies to Recoverin

A. V. Bazhin, O. S. Slepova*, and N. K. Tikhomirova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 4, pp. 416-419, April, 2001
Original article submitted November 21, 2000

Serious changes in the retina, diagnosed as retinal degeneration and uveitis, are observed only in the presence of high titers of antibodies to recoverin (Ca^{2+}-binding protein, a paraneoplastic antigen) in the blood of rabbits. Negligible changes in the retina of rabbits with low antibody titers are detected only by cytohistochemical analysis of the retina. No changes in the retina develop in rabbits intravenously injected with antibodies to recoverin.

Key Words: *recoverin; paraneoplastic antigens; retinal degeneration; uveitis*

Paraneoplastic (onconeural) antigens are proteins normally expressed by nervous tissue systems, while in oncopathology they can be expressed by tumor cells. In the blood these antigens trigger autoimmune reactions, *i.e.* production of autoantibodies, which cross the blood-brain barrier and induce the development of paraneoplastic neurological syndrome [4]. One of such proteins is Ca^{2+}-binding protein recoverin, normally present in the external segments of retinal rods in vertebrate eyes [2] and inhibiting rhodopsin phosphorylation by rhodopsin kinase [9]. In some forms of malignant cell transformation (small-cell pulmonary carcinoma, non-small-cell lung cancer, and some other tumors) recoverin is expressed by tumor cells [6] and triggers autoimmune processes leading to autoimmune degeneration of the retina [7].

It was shown previously that injection of recoverin to Lewis rats induced high titers of antibodies and inflammatory changes in the retina [1,3].

We studied degenerative changes in the retina under the effect of antibodies to recoverin.

MATERIALS AND METHODS

Purification of recombinant recoverin from *E. coli* cells included extraction of cells with EGTA-containing buffer (pH 8.0), precipitation with ammonium sulfate, and hydrophobic chromatography on phenyl sepharose [8]. The purity of the preparation was evaluated by PAAG electrophoresis [5] with subsequent silver staining.

Rabbits were immunized with recombinant recoverin in complete Freund's adjuvant: controls were injected with complete Freund's adjuvant without recoverin. Ophthalmological examination of the fundus oculi was repeated every 3 days. Immune response was recorded by ELISA with recombinant recoverin.

Polyclonal monospecific antibodies to recoverin were prepared as described previously [8] using immobilized recoverin on BrCN-activated sepharose. For cytohistochemical analysis, retinal sections from experimental and control animals stained with hematoxylin and eosin were examined under a light microscope.

Polyclonal monospecific antibodies were injected into the marginal ear vein 3 times with 2-day intervals. The level of antibodies to recoverin was evaluated 1 day after injection by ELISA with recombinant recoverin. Ophthalmological examination of the fundus oculi and retina was carried out every 3 days after the last immunization.

RESULTS

Injection of equal doses of recoverin to rabbits induced different immune responses. After reimmunization, the

A. N. Belozerskii Institute of Physicochemical Biology, Moscow State University; *G. Helmholtz Institute of Eye Diseases, Moscow. ***Address for correspondence:*** tikhomir@usa.net. Tikhomirova N. K.

0007-4888/04/1314-0350$25.00

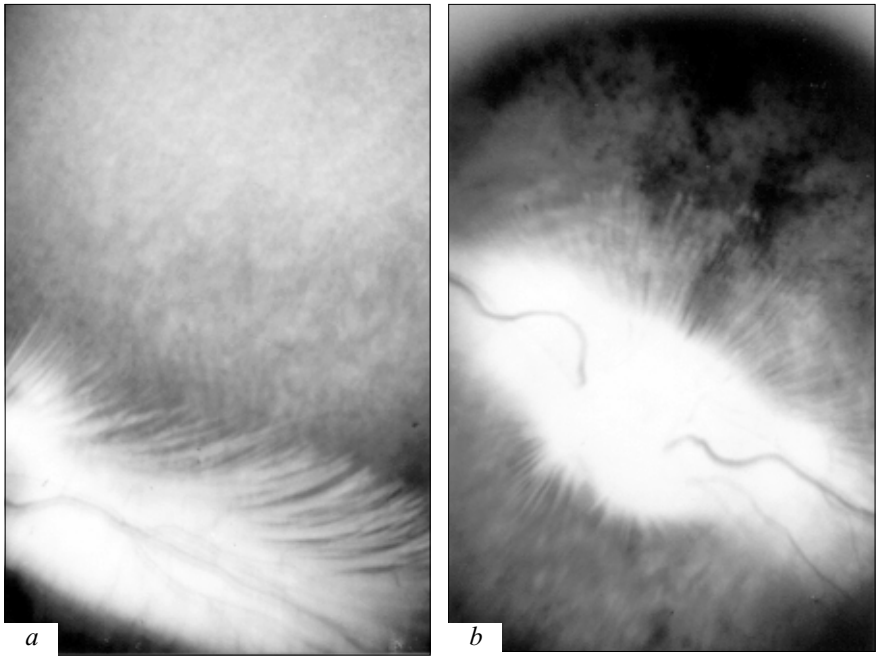

Fig. 1. Fundus oculi in rabbits with low (*a*) and high (*b*) titers of antirecoverin antibodies.

maximum titer of antibody to recoverin in rabbit No. 2 was 1:12,000, while in rabbit No. 1 it was 1:320. In rabbit No. 1, optic media and fundus oculi were unchanged and ophthalmological picture did not differ from that on the day of the first immunization (Fig. 1, *a*). In rabbit No. 2, redistribution of pigment in the retina was paralleled by the appearance of numerous degeneration foci of different size with blurred contours; hemorrhages along narrowed vessels were seen at the retina periphery (Fig. 1, *b*). These changes indicate the formation of chorioretinal foci in the retina of rabbits with high titers of antirecoverin antibodies.

Histological analysis of retinal sections showed changes in the retina even in rabbits with low blood antibody titers (Fig. 2, *a*). Generally, the structure of the retina was intact, all layers were well preserved, but sites of rod and cone disintegration appeared, granular layers were thickened, edema and migration of pigmented epithelium were observed. Histological findings indicated deep morphological changes in the retina of rabbits with high titers of antirecoverin antibodies. The retina was thickened due to accumulation of interstitial fluid in the plexiform layer, internal retinal layers, and in the optic fiber layer; similar disorganization of the internal granular layer and neuroepithelial degeneration, fragmentation of the rod and cone layer, detachment and degeneration of the retinal pigmented epithelium were seen (Fig. 2, *b*). Focal hemorrhages, thrombosis, and stenosis of blood vessels, capillary spasm were seen at the choroid periphery (data not presented). Greater magnification showed neuronal accumulations and death, fragmentation and

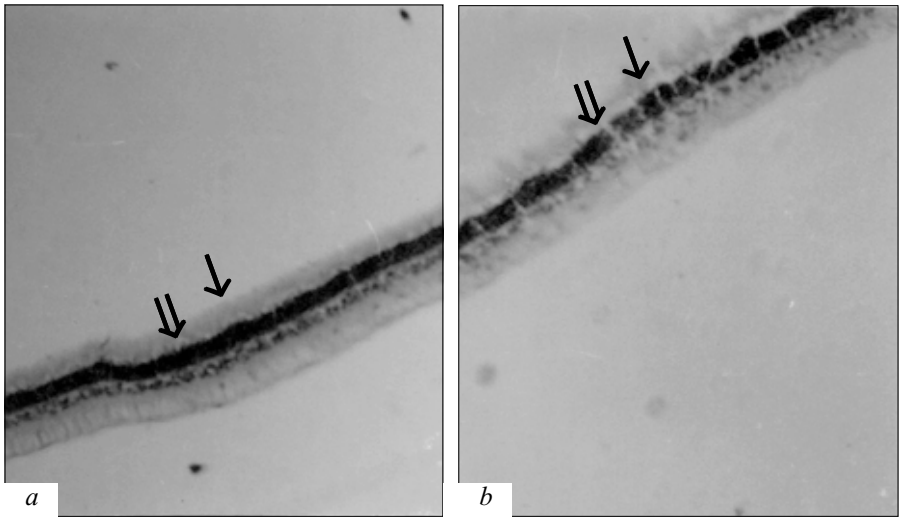

Fig. 2. Retina from rabbits with low (*a*) and high (*b*) titers of antirecoverin antibodies. Here and in Fig. 3: hematoxylin-eosin staining, ×25; arrow shows photoreceptor layer, double arrow shows pigmented epithelium.

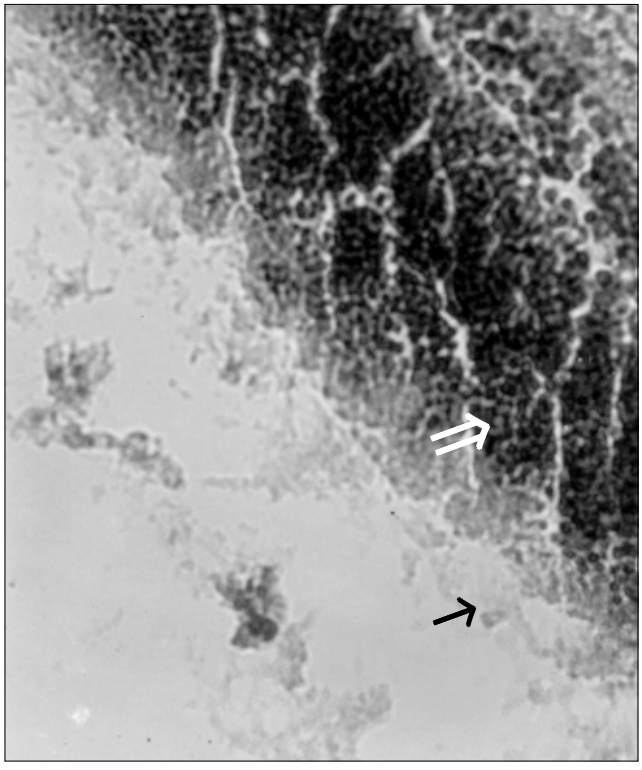

Fig. 3. Retina from a rabbit with high titer of antirecoverin antibodies.

disintegration of rods and cones, release of disks into the subretinal space (Fig. 3).

Hence, induction of uveitis and degeneration of the retina is possible only in the presence of high serum titers of antirecoverin antibodies.

In order to elucidate whether the presence of antirecoverin antibodies in the blood of experimental animals is a sufficient condition for the development of retinal degeneration, polyclonal monospecific antibodies to recoverin were injected into rabbit marginal ear vein to attain the final concentration of injected antibodies in the serum 1:320. However no changes in the retina were detected neither by ophthalmological examination, nor by subsequent histochemical analysis of retinal sections (data not presented). The absence of retinal degeneration in animals injected with antirecoverin antibodies (but not recoverin) is not clear. These data are in line with the results of intravenous transfer of antibodies to paraneoplastic Hu-antigen, which failed to induce the development of the neurological syndrome [10]. It seems to be due to the fact that recoverin-induced retinal degeneration is a complex process involving not only antibodies produced by B cells, but also T cells.

The study was supported by the Russian Foundation for Basic Research (grant No. 98-0449293).

REFERENCES

1. G. Adamus, H. Ortega, D. Witkowska, and A. Polans, *Exp. Eye Res.,* **59,** No. 4, 447-455 (1994).
2. A. M. Dizhoor, S. Ray, S. Kumar, *et al., Science,* **251,** 915-918 (1991).
3. I. Gery, N. P. Chanaud, and E. Anglade, *Invest. Ophthalmol. Vis. Sci.,* **35,** No. 8, 3342-3345 (1994).
4. A. O. Gure, E. Stockert, M. J. Scanlan, *et al., Proc. Natl. Acad. Sci. USA,* **97,** No. 8, 4198-4203 (2000).
5. U. K. Laemmli, *Nature* (London), **227,** 680-688 (1970).
6. A. S. Polans, J. Buszylko, J. Crabb, and K. Palczewski, *J. Cell,* **112,** No. 5, 981-989 (1991).
7. A. S. Polans, D. Witkowska, T. L. Haley, *et al., Proc. Natl. Acad. Sci. USA,* **92,** 9176-9180 (1995).
8. I. I. Senin, A. A. Zargarov, A. M. Alekseev, *et al., FEBS Lett.,* **376,** 87-90 (1995).
9. I. I. Senin, K. R. Dean, A. A. Zargarov, *et al., Biochem. J.,* **321,** 551-555 (1997).
10. P. A. E. Sillevis Smitt, G. T. Manley, and J. B. Posner, *Neurology,* **45,** 1873-1878 (1995).