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Spatiotemporal distribution of 1P1 antigen expression in the plexiform layers of developing chick retina[1]

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ABSTRACT

Changes in the distribution of 1Pl-antigen in the developing chick retina have been examined by indirect immunofluorescence staining technique using the novel monoclonal antibody (MAb) 1P1. Expression of the 1P1 antigen was found to be regulated in radial as well as in tangential dimension of the retina, being preferentially or exclusively located in the inner and outer plexiform layers of the neural retina depending on the stages of development. With the onset of the formation of the inner plexiform layer 1P1 antigen becomes expressed in the retina. With progressing differentiation of the inner plexiform layer 1P1 immunofluorescence revealed 2 subbands at E9 and 6 subbands at E18. At postnatal stages (after P3) immunoreactivity was reduced in an inside-outside sequence leading to the complete absence of the 1P1 antigen in adulthood. 1P1 antigen expression in the outer plexiform layer was also subject to developmental regulation. The spatio-temporal pattern of 1P1 antigen expression was correlated with the time course of histological differentiation of chick retina, namely the synapse rich plexiform layers. Whether the 1P1 antigen was functionally involved in dendrite extension and synapse formation was discussed.

Key words: *chick retina, development, immunofluorescence, plexiform layer*

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INTRODUCTION

The development of functional neuronal networks of higher vertebrates is based on a strictly controlled sequence of biological phenomena such as cell proliferation and differentiation, neurite outgrowth and synaptogenesis[1,2]. The molecular mechanisms underlying these events have been only scarcely delineated partly due to the complexity of the systems and partly to the minute amount of embryonic material available for analysis. One approach to circumvent such technical restrictions would be the application of the novel hybridoma technology providing monoclonal antibodies for biochemical and biological analysis of rare components within embryonic neuronal tissue[3].

Recently it has been very advantageous achieving access to the monoclonal antibodies for analysis of neuronal histogenesis. Using the monoclonal antibody 2A1 in histological sections, it was found that the 2A1 antigen is a membrane associated cytoskeleton protein and is expressed preferentially in outgrowing axons of retinal ganglion cells [4].

Moreover, the C4 antigen was found to be expressed specifically in the optic fiber layer of the chicken retina[5]. Further analysis has identified the antigen to be an axon-specific cell surface glycoprotein functioning as an adhesion molecule which is essential for directed outgrowth of ganglion cell axons[6].

In this study, we used a new monoclonal antibody to reveal the changes in distribution of 1P1 antigen in the developing chick retina. It is possible to provide a theoretical basis for further analytical approach concerning the biological aspects of the 1P1 antigen on one hand and the principles of neural histogenesis on the other.

MATERIALS AND METHODS

Antibody Generation

Tectal glycoconjugates from E10 chick embryos were purified by affinity chromatography employing a Concanavalin A matrix. These glycoconjugates were used as immunogens for three inoculations of Balb /c mice over a period of 4 wk[5]. After the final booster injection, splenic cells were fused with myeloma cells NS-1 and cultivated in microtiter plates for 2-5 wk[7]. The hybridoma culture supernatants were screened on paraformaldehyde fixed cryostat sections of chick eyes of different developmental stages.

Tissue Preparation

Eyes were removed from white leghorn chickenaged between embryonic day 4 (E4) and adulthood. Eyes were fixed in 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4) for 1 d at 4℃, washed with phosphate buffered saline (PBS) and cryoprotected in 25% sucrose overnight at 4℃. Thereafter the eyes were embedded in OCT compound on dry ice and cryosectioned into 10-15 μm sections. The plane of sectioning was perpendicular to the optic fissure (horizontal). Sections were mounted onto gelatin coated slides.

Immunofluorescence Staining

Frozen sections were preincubated in 0.1% BSA at 25℃ for 30 min, and then incubated with MAb 1P1 (1:1000) at 4℃ for 15 h. After washing for 1 h, sections were incubated with FITC-goat

anti-mouse IgG (1:75, Jackson Immuno Research Lab.) for 2 h at 25° C. After final washing, sections were mounted in Elvanol solution. In control sections MAb 1P1 was replaced by BSA.

RESULTS

The developmental regulation of 1P1 antigen expression was analyzed in radial and tangential dimension of the chicken retina using indirect immunofluorescence staining.

Fig 1 depicted the changes in the distribution of the 1P1 antigen in a series of horizontal frozen sections at central locations near the fundus of retinae from E4 through adulthood. At E4/E5 very faint immunoreactivity was observed in the inner (more vitreous) part of retina. At E6.5 a diffuse staining was found in the vicinity of perikarya of primitive ganglion cells (Fig 1B). At this stage the retina was composed only of a relatively uniform neuroblast and a ganglion cell layer (Fig 1A). Progressively, staining became concentrated in the primitive plexiform layer. The 1P1 antigen was detected in the inner plexiform layer (IPL) for the first time at E7 (Fig 1C, D) and in the outer plexiform layer (OPL) at E9 (Fig 1E). With proceeding maturation of the retina, MAb 1P1 revealed 3 to 6 more intensely stained subbands in the IPL which increased considerably in width (Fig 1E-L) until postnatal day 3 (P3). The number of subbands in the IPL increased with time: 2 subbands at E9 /10, 3 at E12/15, 5-6 at E18/E21 and 6-7 at P3. Alter P3 immunoreactivity in the IPL gradually diminished and was essentially absent in the inner sublayers of the IPL at P10 (Fig 1M) and at P30 in the outer sublayers (Fig 1N). In adulthood the 1P1 antigen could not be detected in any of the sublayers of the IPL of retina (Fig 1O).

The dynamic regulation of 1P1 expression in the OPL was somewhat different from that in the IPL. Starting from E9, 1P1 immunoreactivity became more pronounced with further tissue differentiation revealing at least 2 subbands in the OPL. In addition, staining extended partially into the photoreceptor layer. This staining pattern was found to be qualitatively similar in adulthood.

In a series of horizontal sections the 1P1 antigen distribution was analyzed in the circumference of the whole eye ball. Fig 2 depicted the staining pattern at E7, E9, E12 and E14. At E7 1P1 immunoreactivity was located in the presumptive IPL at the near-central area of the retina slightly temporal to the optic fissure. With progressive development the 1P1 staining extended towards the periphery of the retina. At E9 staining in the vicinity of the ora serrata was observed in the ventro-temporal region of the retina, whereas the appearance of 1P1 antigen was delayed at the dorso-nasal margin of the retina. By E12 MAb 1P1 marked the IPL completely. These results indicated that 1P1 antigen expression followed a central-to-peripheral sequence. The spatial sequence of 1P1 immunoreactivity in the OPL showed a similar pattern as that in the IPL, but with a delay of 2 days, i.e. it appeared in the central area of the retina around E9 and spread to the ora serrata at around E14.

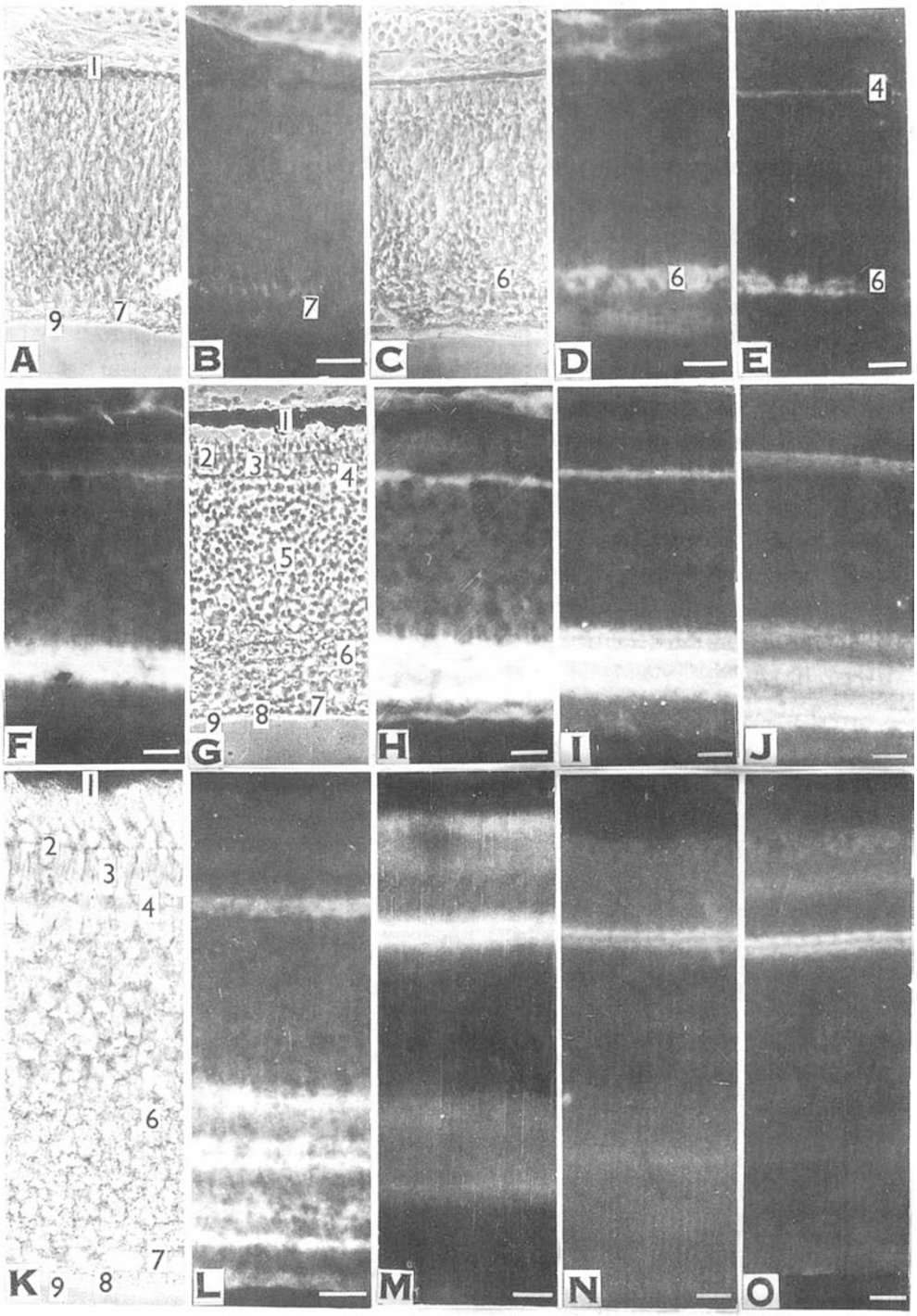
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Fig 1. ◁Developmental regulation of 1P1 antigen expression in chick retina. Paraformaldehyde fixed cryosections were immunolabeled with monoclonal antibody 1P1 and a secondary fluorescence labeled antibody. The plexiform layers of the retina were specifically marked by MAb 1P1, especially during the period of dendritic growth and synapse formation. Phase contrast: A, C, G, K; immunofluorescence: B, D, E, F, H, I, J, L, M, N, O. Developmental stages: Embryonic day (E) 6:A, B; E7: C, D; E9: E; E12: F; E15: G, H; E18: I; E21: J; postnatal day (P) 3: K, L; P10: M; P30: N; adult: O. Number indication: 1, pigment epithelium; 2, outer limiting membrane; 3, outer nuclear layer; 4, outer plexiform layer; 5, inner nuclear layer; 6, inner plexiform layer; 7. ganglion cell layer; 8. optic fiber layer; 9, inner limiting membrane. Scale: 25 μm.

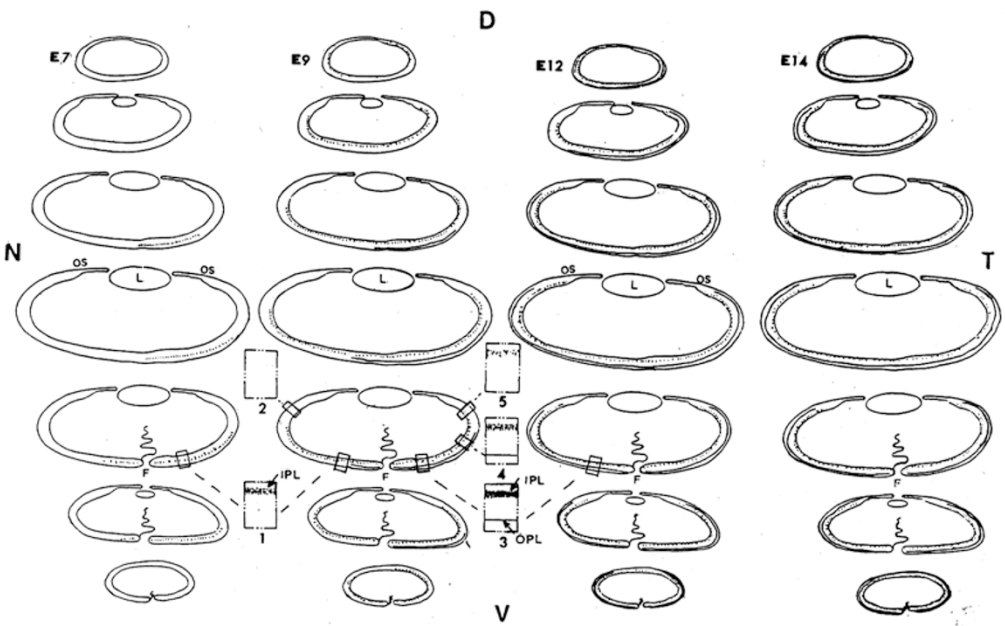

Fig 2. Spatial distribution of 1P1 antigen expression in the whole retina of different stages of development. Schematic drawings from MAb 1P1 immunostained series of horizontal sections of retinae from E7, E9, E12 and E14. The rectangle insets 1-5 represent enlargements of immunostainings of central(1,2), medial(4) and peripheral(2,5) region of the retina. The bands shown in insets reflect the width and staining intensity of the IPL and OPL, respectively. 1P1 immunoreactivity spreads from central to peripheral regions of the retina in the IPL between E7 and E12, in the OPL between E9 and E14. The staining intensity decreases from central to peripheral regions as illustrated in insets 1-5. Abbreviations: D, dorsal; F, optic fissure; L, lens; N, nasal; OS, ora serrata; T, Temporal; V, ventral.

The qualitative regulation of 1P1 antigen expression in inner-outer and centro-peripheral dimensions of the retina was also subject to quantitative changes. In horizontal sections of E9 retinae the staining intensity in the IPL at the near-central area was stronger than that in the middle-peripheral region (Fig 2). In addition, at

E9 the IPL revealed a more pronounced immunoreactivity than the OPL (Fig 1E). The disappearance of 1P1 staining exhibited a similar central-peripheral and inside-outside sequence, i.e. staining faded first in the inner part, then in the outer part of the IPL. Comparing IPLs at different stages of development, the immunofluorescence staining was most intense at E15 (Fig 1H). In OPLs the highest immunoreactivity was observed much later in development at P10 (Fig 1M).

DISCUSSION

The present study has revealed that the 1P1 antigen expression began at about E7 in the IPL and at E9 in the OPL, and spreads from central regions (fundus) to the periphery (ora serrata) with a delay of 5 d. This spatiotemporal sequence was strictly correlated with the differentiation of plexiform layer in the retina[8]. Most interestingly, in both plexiform layers 1P1 antigen expression was subjected to highly dynamic regulation with maximal immunoreactivity found during the developmental period of dendrite extension and synapse formation. Later in development the antigen expression ceased, although more pronounced in the OPL, since in adulthood no antibody binding could be observed at IPL.

These data strongly suggested that the 1P1 antigen might be involved in developmental aspects of dendritic outgrowth and synapse formation. This working hypothesis was further substantiated by preliminary data indicating that the 1P1 antigen was associated with the extracellular matrix (Schlosshauer, unpublished). Therefore, the antigen could function as a substratum component like laminin or collagen to modify neurite outgrowth[9].

Both plexiform layers were formed by neurites from different retinal neurons-cell perikarya were essentially absent from the IPL and OPL. In the IPL dendrites of ganglion-bipolar and amacrine cells formed a dense synaptic network but not in the case of photoreceptors or horizontal cells[10]. In addition, ganglion cells send only dendrites but no axons into the presumptive IPL[11] . Currently we have been investigating the molecular mechanisms that allowed such precise outgrowth of different neurite type. One hypothesis postulated that different cell surface and extracellulax matrix molecules that were permissive or inhibitory for one neurite type versus the other. Ganglion cell axons for example could be inhibited from growing into the presumptive inner plexiform layer by virtue of an unknown inhibitory protein in this layer, whereas in the optic fiber layer/inner limiting membrane, the laminin provided an excellent substratum for outgrowing axons[12]. This might explain why ganglion cells extend exclusively into the optic fiber layer.

Similar molecular mechanisms could prevent the ganglion cell dendrites to grow into the optic fiber layer and /or attract dendrites selectively into the inner plexiform layer. Molecular components involved in such mechanisms would be postulated to be locally and possibly temporally restricted. Consequently we aimed to identify monoclonal antibodies that labeled only plexiform layers and do achieve this in a

developmentally dynamic fashion. MAb 1P1 is a member of this class and to our knowledge the first antibody showing these characteristics. Therefore it is believed that MAb 1P1 may be the best candidate specific for an instructive antigen with respect to directed neurite outgrowth.

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