able that *A* cells can be transmuted into *B* cells by loss of the enzymatic activities necessary for the formation of α-MSH. This type of change has been suggested for the PI cells of the trout, which in vitro appear to acquire the ability to secrete increasing amounts of bioactive ACTH while their α-MSH secretion decreases (although alternative explanations are possible) (*17*). Although the *B* cells resemble PD corticotrophs, it is possible that appropriate stimuli could cause them to cleave ACTH to α-MSH and CLIP, as *A* cells evidently do. Further, their functional regulation may differ from that of PD corticotrophs owing to their location in the avascular PI, which receives dopaminergic and serotonergic fibers from the brain (*18*), whereas the PD corticotrophs are devoid of a nerve supply and are controlled mainly by factors from the hypothalamus that reach them through the hypophysial portal vessels. Studies of the responses of separated *A* and *B* cells to various agents in vitro would therefore be of great interest.

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19. Porcine and ovine ACTH(1–39) were provided, respectively, by J. Ramachandran, University of California, and Ciba-Geigy. We thank A. E. Wallen, Sr., for his gift of the dogs. Supported by PHS grants AM-02463, NB-02893, and T32-AM-0727, Research Career Award AM-13,576 (to N.S.H.), National Research Service Award F-32-AM-06261 (to M.E.P.), and the Lita Annenberg Hazen Charitable Fund.

4 August 1980; revised 22 September 1980

Immunophagocytic Properties of Retinal Pigment Epithelium Cells

Abstract. *Retinal pigment cells were dislodged from normal monkey eyes and incubated in glass-slide chambers. All viable pigment cells adhered strongly to glass. They demonstrated surface receptors for the Fc portion of immunoglobulin G and for the third component of complement by selectively binding and phagocytizing antibody or complement-coated erythrocytes. These phagocytic cells with receptors were identified as retinal pigment cells by characteristic ultrastructural features. Thus, retinal pigment cells, which are generally believed to be derived from neural tissue, are not only scavengers of photoreceptor cell debris, but also have surface receptors and phagocytic functions that may be important in ocular defense.*

The retinal pigment epithelium (RPE) consists of a monolayer of cells interposed between the rod and cone photoreceptor elements of the sensory retina and the choroidal blood circulation. As a component of the blood-retina barrier (*1*, *2*), the neuroectoderm-derived (*3*, *4*) RPE participates in processes essential to photoreceptor cell homeostasis (*1*, *2*). Among the functions of the RPE is the phagocytosis and intracellular lysosomal degradation of aged photoreceptor membranes that are shed from the tips of rod and cone cells in a diurnal cycle (*5–7*). The phagocytic nature of the RPE (*8*, *9*) led us to investigate whether specific receptor mechanisms known to mediate the attachment, ingestion, and elimination of particulate matter by other phagocytes might also be demonstrable in RPE cells. In particular, glass-adherent macrophages of bone marrow origin have surface receptors for the Fc region of immunoglobulin G (IgG) antibody and for the third component of complement, both of which bind and thereby promote the phagocytosis of specifically coated particles (*10*, *11*). We therefore used erythrocytes coated with IgG and complement-coated erythrocytes to demonstrate receptor-mediated binding and phagocytosis by RPE cells.

Suspensions of retinal pigment cells were prepared from *Macaca fasicularis*

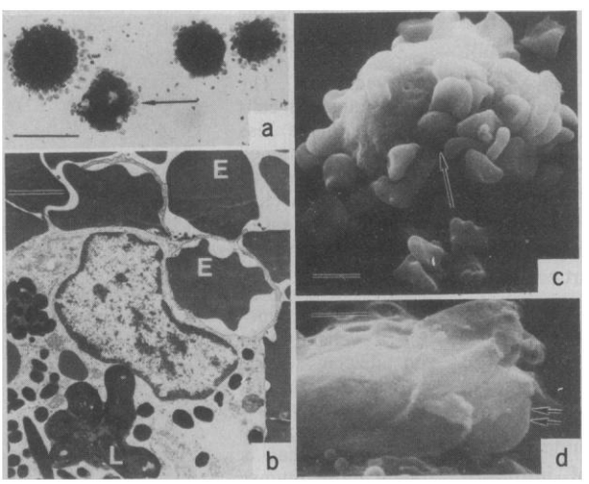

Fig. 1 (a) Photomicrograph of EA rosettes. Deeply pigmented RPE cells obtained from *M. fasicularis* have bound human erythrocytes coated with IgG antibodies to red blood cells. In one cell (arrow), pigment granules appear to be displaced by phagocytized erythrocytes. Scale bar, 50 μm. (b) Transmission electron micrograph of a portion of a RPE cell from *M. fasicularis*. The pigment cell has bound IgG-coated erythrocytes (*E*). Tight attachment has caused many of them to become deformed. Two erythrocytes are apparently being engulfed by the RPE cell which exhibits typical features such as round and elliptical melanin granules, combined bodies, and a lamellar figure (*L*) believed to represent rod or cone (or both) membrane stacks within a secondary lysosome. Scale bar, 2 μm. (c) Scanning electron micrograph of a RPE cell EA rosette. Many of the IgG-sensitized red blood cells bound to this *M. fasicularis* RPE cell are being engulfed by membrane ruffles. A recently phagocytized erythrocyte may be seen just beneath the cell surface (arrow). Scale bar, 5 μm. (d) Higher magnification of a sensitized erythrocyte (arrows) undergoing phagocytosis by a RPE cell. Scale bar, 2 μm.

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and *M. mulatta* eyes by a procedure similar to that proposed by Heller (*12*). In brief, we removed the anterior ocular segment and the vitreous body, peeled the neural retina from the retinal pigment monolayer, and gently brushed the RPE cells into Hanks balanced salt solution (HBSS) with a soft brush. Intact pigment cells were collected by centrifuging the initial suspensions for 10 minutes at 90*g*. The supernatant was discarded and the RPE cells were resuspended in HBSS and placed in Tissue-Tek glass-slide chambers. We found that the viable pigment cells attached to the slide chambers during 2-hour incubations at 37°C. The attached pigment cells were overlaid with a 0.5 percent suspension of fresh erythrocytes coated with IgG antibody (EA) (*13, 14*) or with immunoglobulin M (IgM) antibody and complement components (EAC) (*15*). After 30 minutes, red blood cells not bound to RPE cells were removed by washing four times with HBSS, and the preparations were fixed in 2.5 percent buffered glutaraldehyde. Some cultures were prepared for scanning electron microscopy and some for transmission electron microscopy (*14*). Other preparations were stained with hematoxylin and eosin and scanned to determine the number of cells with Fc and complement receptors; these cells were identified by the binding of EA and EAC in rosettes around the pigment cells (Fig. 1a).

In multiple experiments, about 85 percent of the glass-adherent retinal pigment cells formed EA rosettes (Table 1). This binding was shown to be specific for the Fc region of the IgG on EA. In control experiments, uncoated red blood cells and erythrocytes sensitized with the $F(ab')_2$ fragments of IgG antibody (*16*) failed to bind to the RPE cells. Excess soluble IgG not attached to erythrocytes markedly inhibited EA rosette formation by competing for the Fc receptor sites on the RPE cells. Excess soluble $F(ab')_2$ fragments did not inhibit rosette formation. Eighty-four to 91 percent of the pigment cells formed EAC rosettes when the cells were exposed to erythrocytes coated with IgM-activated complement components from nonlytic mouse serum deficient in the fifth component of complement (C5). This binding was not inhibited by excess soluble IgG. Red blood cells treated with IgM antibody or with C5-deficient serum alone did not form rosettes. We conclude therefore that the EAC-rosette binding was mediated by complement receptors located on the RPE cell surface.

Transmission electron microscopy of pigment cell-erythrocyte rosettes (Fig. 1b) revealed that all of the cells could be identified as typical RPE cells. The cells possessed characteristic RPE features such as elliptical and round melanin granules, lipid inclusions, secondary lysosomes containing large lamellar inclusions that were presumably derived from engulfed membrane stacks of rod or cone cells (or both), and residual and combined bodies (*4*). Direct contact between pigment cell membranes and attached red blood cell membranes was clearly seen. The RPE cells demonstrated a number of macrophage-like properties when RPE cell-erythrocyte rosettes were viewed by scanning electron microscopy (Fig. 1, c and d). Included among these were the binding and phagocytosis of IgG and complement sensitized erythrocytes and the presence of membrane ruffles or microvilli (or both).

Table 1. Percent of retinal pigment epithelium cells with immune receptors. Glass-adherent cells of retinal pigment epithelium, isolated from monkey eyes within 30 minutes after the animals were killed, were used to form EA and EAC rosettes. Human erythrocytes were coated with one-half of the least agglutinating titer of IgG antibody or $F(ab')_2$ fragments (*14, 16*). Complement-coated sheep erythrocytes were prepared by sensitization with one-fourth of the least agglutinating titer of IgM antibody followed by C5-deficient AKR mouse serum diluted to a final concentration of 1:20 (*15*).

Source of cells	Coating of test erythrocytes	Displacing reagent*	Percent of cells forming rosettes†
	Macaca fasicularis		
1	Uncoated	None	0.5
	IgG	None	87.0
	IgG	IgG	9.0
	$F(ab')_2$	None	1.0
2	IgM and complement	None	86.0
	IgM	None	6.5
3	Uncoated	None	2.0
	IgG	$F(ab')_2$	91.5
	$F(ab')_2$	None	3.5
	IgM and complement	None	84.5
	IgM	None	1.0
	IgM and complement	IgG	90.0
	Macaca mulatta		
1	Uncoated	None	6.0
	IgG	None	83.0
	IgG	IgG	4.5
	$F(ab')_2$	None	9.0
2	Mouse serum	None	0.0
	IgM and complement	IgG	91.0
	IgM	None	2.5
3	IgG	None	84.5
	IgG	IgG	3.0
	IgG	$F(ab')_2$	84.5
	$F(ab')_2$	None	0.0
	IgM and complement	IgG	91.5
	IgM	None	3.0

*In competition experiments, dissolved rabbit antiferritin IgG at 1 mg/ml or $F(ab')_2$ fragments (*16*) at 2 mg/ml in erythrocyte suspensions were used as displacing reagents. Incubations lasted 30 minutes at 25°C. †Retinal pigment cells were considered to have receptors if more than four erythrocytes were attached. At least 200 cells were counted in all samples.

Our experiments revealed that RPE cells possess features that are characteristic of mesenchyme-derived macrophages. The demonstration of complement- and IgG-mediated adherence and phagocytosis indicates that the retinal pigment epithelium, though widely believed to be of neural origin, has the capacity to express immunologic functions. The presence of such properties in RPE cells isolated from normal monkey eyes implies an active role for the RPE in ocular defense which corresponds to the location of the pigment cell monolayer between the systemic circulation and the neural retina. Other investigators have suggested, on the basis of morphological studies, that the freely migrating, pigmented phagocytes seen in a variety of retinal lesions originate from proliferating RPE cells (*17, 18*). These observations, taken together with our findings, would lend strong support to the contention that the RPE actually comprises a population of specialized central nervous system macrophages that are probably distinct in origin from blood-borne mononuclear phagocytes. Finally, the detection of specific receptors mediating attachment and phagocytosis of particulate matter by the RPE also suggests that impaired recognition processes involving RPE cell receptors might also be the basis for some forms of retinal dystrophy in which photoreceptor cell debris is not efficiently eliminated (*9, 19–21*).

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12 March 1980; revised 7 July 1980

Resource Partitioning During Reproduction in the Norway Rat

Abstract. *Rat pups nursed by pregnant dams grow as fast as pups reared by dams that are not pregnant. Moreover, litters that were in utero during a lactation are as numerous at birth and grow as fast as pups developing in a nonlactating, pregnant mother. These litters continue to grow as fast as pups born to nonlactating dams whether or not the first litter remains after the birth of the second litter. When pregnant and lactating dams have a restricted food supply, some dams are capable of extending the duration of their pregnancies by over 2 weeks past that of nonlactating, pregnant dams. This facultative prolongation of pregnancy apparently allows females to carry normal litters to term.*

Mothers should be expected to partition time and energy between themselves and their offspring in such a way that the number of potential descendants is maximized. In this report we describe the ability of dams to partition their energy resources between themselves and their offspring during a concurrent lactation and pregnancy.

Lactation demands enormous energy from mother rats (*1*) and, since rats can become impregnated during a postpartum estrus (*2*), the demands of a concurrent pregnancy may intensify the strain of lactation. In our study, we determined whether the dams that were both pregnant and lactating would pass less energy, as measured by pup weight, to either the litter they were nursing or to the junior litter in utero than if they were simply pregnant or lactating.

Eight primiparous Wistar rat mothers were impregnated on the evening after the dams gave birth. Each day, maternal body weight, senior litter pup weight, and maternal food intake were recorded between 0900 and 1200 hours. In addition, the duration of the postpartum pregnancy and the number and the daily weight change of the pups born in the junior litters were also recorded. These data were compared, where appropriate, to those obtained in a similar manner from groups of eight mothers that were either pregnant for the first time, lactating for the first time, or lactating for the second time in their lives (*3*).

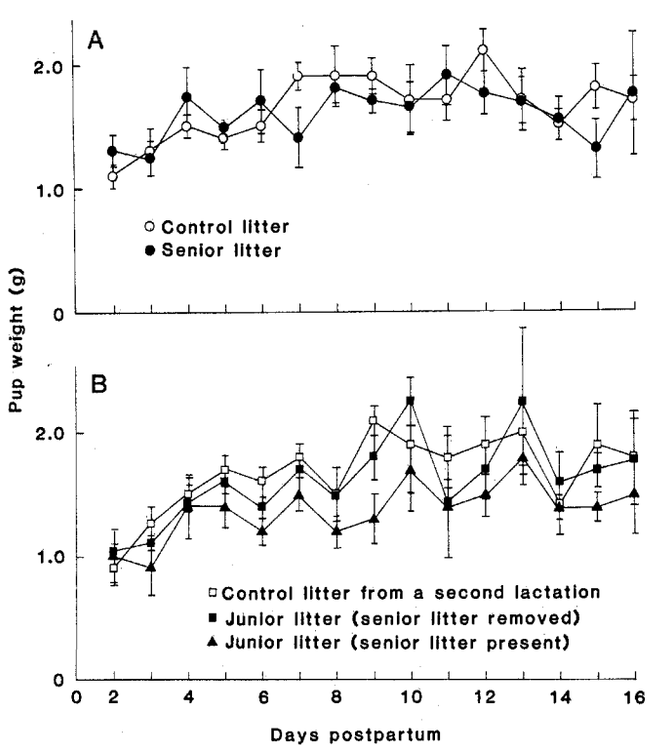

Fig. 1. (A) Mean daily change in individual pup weight of senior litter nursed by a pregnant dam compared to that of pups nursed by a nonpregnant mother (control). (B) Mean daily change in individual pup weight of junior litters nursed by dams with or without the senior litters present compared to dams rearing their second litters that were carried to term while the mothers were not lactating.

Senior litters of eight pups that were nursed by pregnant mothers grew as fast as pups reared by nonpregnant dams [$F(1, 18) = 0.57$, $P > .05$] (Fig. 1A). The number of pups born to pregnant and lactating dams were equal in number and weight to those born of nonlactating dams ($U = 31.5$, $P > .05$; $t = 1.04$, $P > .05$). Whether or not the senior litter was allowed to remain with the dam after she gave birth to the junior litter, the junior litter weight gain was not significantly less than pups raised by dams nursing pups in their second lactation (Fig. 1B) [$F(2, 25) = 3.20$, $P > .05$]. Mothers, therefore, can support the simultaneous normal growth of both senior and junior litters.

We considered the possibility that mothers could provide the resources to nurture junior litters with no apparent cost to senior litters by catabolizing their own tissues to supply energy to the young, as the dams do when their food supply is restricted (*4*). The change in body weights of dams that were pregnant and lactating, however, was no different from that of control pregnant females over the course of their pregnancy ($t = 0.54$, $P > .05$) or during the first 3 weeks of lactation without pregnancy ($t = 1.25$, $P > .05$).

Another mechanism that would allow dams to compensate for the additional energy required during a pregnancy accompanied by lactation would be an increase in food intake above that of lactating dams (*5*). Mothers that were pregnant and lactating, however, did not eat any more than mothers that were only lactating [$F(1, 18) = 0.91$; $P > .05$], at least during the first 16 days postpartum, which includes the period of peak milk production (*6*).

A third mechanism may postpone the investment of energy in the in utero litter until the lactational investment has peaked; resources previously directed to milk production could then be transferred to support the pregnancy. In our study, the time between impregnation and parturition was extended by about a week (Fig. 2). Previous studies have shown that rats have a delay in uterine implantation in a postpartum pregnancy and that this may be proportional to the size of the senior litter (*7*). While a delay in implantation of the junior litter delays the investment of energy to those pups,

0036-8075/81/0102-0076$00.50/0