pressor function was demonstrated on one of three occasions.

Cells from lupus patients were incubated with calf thymosin fraction 5 (*6*) or CTE (*7*) for 1 to 7 days, washed, irradiated, and added to an MLC reaction (Table 1). After incubation with thymosin (250 μg/ml) for 24 to 48 hours, cells from 7 of 12 patients with SLE significantly suppressed the MLC reaction; in two patients suppressor activity was not induced until cells were exposed to thymosin (500 μg/ml). Culture of SLE cells with CTE for 7 days led to the generation of suppressor cells in four of seven patients; in patient J.M., exposure to CTE led to the induction of a significant helper effect (*8*).

Our results indicate that SLE patients lack suppressor function. We do not feel that the lack of suppressor function in SLE is due to excess helper activity since dilutional studies with Con A–activated lupus cells did not demonstrate suppression of the MLC reaction. Abdou *et al.* (*9*) and Breshnihan and Jasin (*10*), using different assay systems, reported that suppressor function was depressed in SLE, but returned toward normal as the activity of the disease declined. However, in our studies suppressor function was absent in 14 consecutive SLE patients, including three patients with inactive disease who had not received any drug therapy for more than 6 months and three other patients with inactive disease who had been treated with steroids or immunosuppressive agents (or both). We also found that suppressor function could be induced in SLE cells after incubation with thymosin or CTE, suggesting that thymic manipulation may be a useful therapeutic modality in some cases of SLE.

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12. Supported by grants from NIH (HD 07778, AI 11576) and NCI (CA16964), by PHS training grant ST01 4M 05582-08, and by the University of Wisconsin Medical School Research Committee (G-491-26), the University of Wisconsin Graduate School Research Committee (170859), the John A. Hartford Foundation, Inc., Hoffman–LaRoche, Inc., and (to H.S.W.) the Deutsche Forschungsgemeinschaft. This is paper number 122 from the Immunobiology Research Center.

1 March 1977; revised 26 April 1977

Defective Phagocytosis of Isolated Rod Outer Segments by RCS Rat Retinal Pigment Epithelium in Culture

Abstract. *Retinal pigment epithelium cultured from normal rats phagocytizes large amounts of rod outer segment fragments isolated from normal rats and from RCS rats with inherited retinal degeneration. Cultured RCS rat pigment epithelium rarely ingests outer segment material, although the cells extend cellular processes around fragments of either type. Both normal and RCS pigment epithelium phagocytize polystyrene spheres. This demonstrates that RCS rat pigment epithelial cells contain a defect in the mechanism for phagocytizing outer segments.*

Phagocytosis of the tips of rod outer segments by the retinal pigment epithelium occurs as part of the outer segment renewal process (*1*). A balance is thereby established between the synthesis of new disks at the base of the outer segment (*2*) and removal at the distal ends. In the RCS rat with inherited retinal degeneration, phagocytosis of outer segment tips by the pigment epithelium fails to occur in vivo (*3–5*), resulting in the accumulation of membranous outer segment debris in the subretinal space and degeneration of the photoreceptor cells (*4–6*). Phagocytosis is not totally defective, since RCS rat pigment epithelial cells are capable of ingesting carbon particles injected into the subretinal space (*7*).

The pigment epithelium has been shown to be the primary site of expression of the mutant RCS gene in chimeras formed by combining embryos of a normal and an RCS rat. Accumulation of membranous debris and loss of photoreceptor cells occurred only opposite the RCS pigment epithelium in the chimeras, while photoreceptor cells with outer segments in contact with normal pigment epithelium were unaffected (*8*). However, it has not been demonstrated how the RCS gene is expressed in the pigment epithelium. The RCS pigment epithelial cell may have a defective mechanism for phagocytizing outer segment material, or the RCS pigment epithelium may prevent phagocytosis by adversely influencing outer segment differentiation or altering outer segment membrane properties. In the study described herein we compared the ability of cultured normal and

RCS rat pigment epithelium to phagocytize either polystyrene spheres or isolated outer segment fragments, and obtained direct evidence that the RCS pigment epithelium contains a defect in the mechanism by which outer segment material is phagocytized.

Primary explant cultures of pigment epithelium were prepared (*9*) from 7-day-old normal pigmented Long-Evans rats (Charles River Breeding Laboratories) or pigmented RCS-p$^+$ rats (*10*). Rod outer segment fragments, prepared from dark-adapted 25-day-old Long-Evans or RCS-p$^+$ rats (*11*), or 1.1-μm polystyrene (Latex) spheres (*12*) were added to pigment epithelium explants on day 4 in vitro. After the cultures were incubated for 1 to 18 hours at 37°C in 5 percent CO_2 and 95 percent air under ambient light the medium was removed and the cultures were fixed and prepared for electron microscopy (*13*).

Pigment epithelial cells cultured from normal and RCS rats phagocytize polystyrene spheres within 1 hour. After 2 hours of incubation, profiles of most cells in sections through a RCS pigment epithelial culture contain several spheres (Fig. 1), similar results being obtained with cultured normal pigment epithelium. However, a marked difference exists between the ability of cultured normal and RCS pigment epithelium to phagocytize isolated rod outer segment fragments. Normal pigment epithelial cells begin to phagocytize outer segment fragments within 1 hour of incubation, and after 2 hours most cell profiles in randomly selected sections of a culture contain several phagosomes when the cells have been incubated with RCS outer segments (Fig. 2a) or with normal outer segments. In contrast, most RCS pigment epithelial cells in culture do not phagocytize outer segment fragments, although an occasional cell profile is found in each culture which contains one or two phagosomes when the cells have been incubated for 2 hours with either a normal or RCS outer segment preparation. The RCS pigment epithelial cells extend microvillous processes into and around outer segment material, but the material is only rarely phagocytized (Fig. 2b). Serial sections reveal that all of the outer segment material in Fig. 2b is contiguous with the extracellular space. After 18 hours of incubation with normal or RCS outer segment preparations, outer segment material continues to be surrounded by microvillous processes but is not phagocytized; as at 2 hours, an occa-

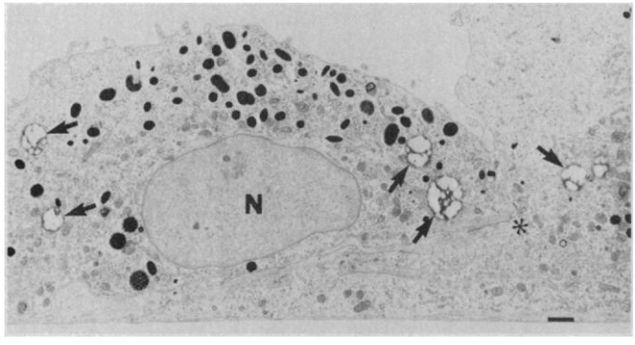

Fig. 1. Cultured RCS rat pigment epithelium incubated for 2 hours with polystyrene spheres contains several phagocytized spheres (arrows). An apical tight junction (*) joins two adjacent cells. Dense melanin granules are present in the apical cytoplasm above the nucleus (*N*). Scale bar, 1.0 μm.

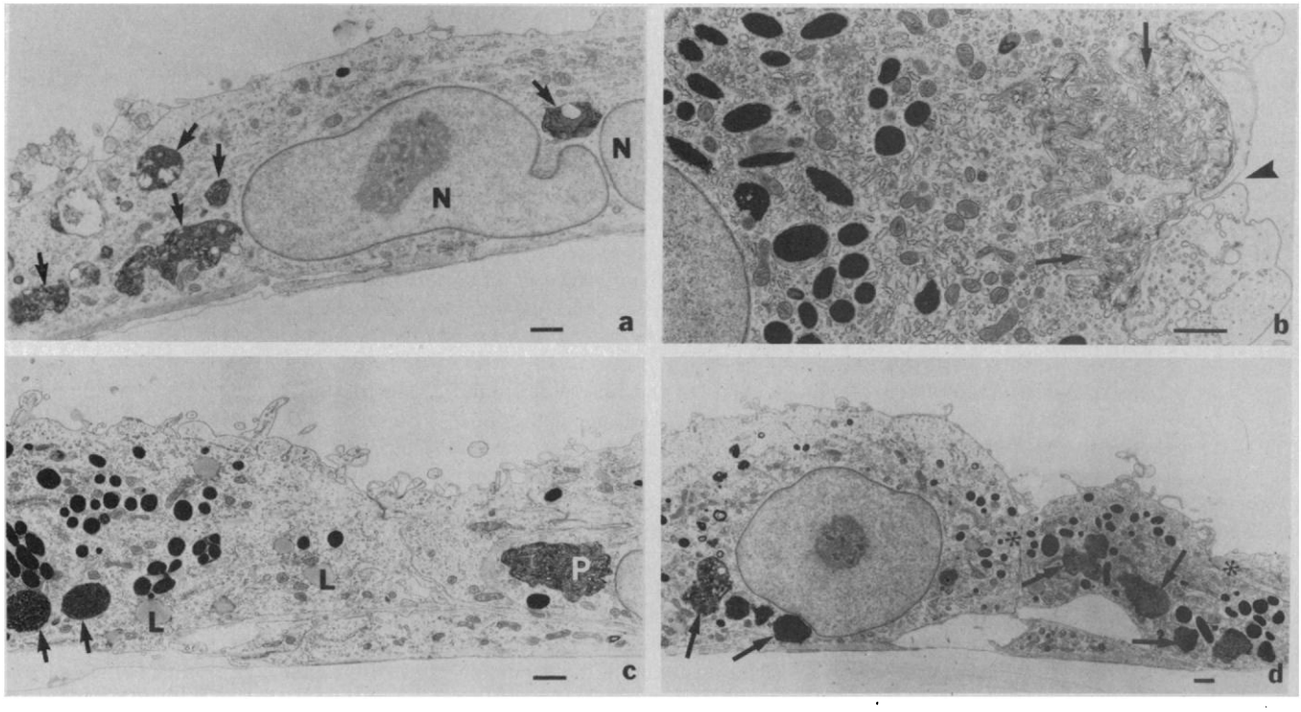

Fig. 2. Cultured rat pigment epithelium incubated with isolated outer segments. (a) A binucleate (*N*) normal pigment epithelial cell incubated for 2 hours with RCS outer segments has several phagosomes containing outer segment material (arrows). (b) Microvillous processes envelop outer segment material (arrows) in cultures of RCS pigment epithelium incubated for 2 hours with normal outer segments. Note that the outer segment material is contiguous with the extracellular space by way of the narrow channel indicated by the arrowhead. Similar results were obtained when cultured RCS pigment epithelium was incubated with RCS outer segments. (c) An occasional cell in each culture of RCS pigment epithelium incubated for 18 hours with normal outer segments contains a phagosome (*P*), although many cells continue to extend microvillous processes around outer segment material. Large premelanosomes (arrows) and lipid droplets (*L*) are seen in cultured pigment epithelium. (d) Phagocytized outer segments (arrows) are found in normal pigment epithelial cells incubated for 18 hours with normal outer segments. Adjacent pigment epithelial cells are joined by apical tight junctions (*). Similar results to those of (c) and (d) were obtained when cultures were incubated with RCS outer segments. Scale bars, 1.0 μm.

sional RCS pigment epithelial cell profile in each culture may contain one or two phagosomes (Fig. 2c). Under the same conditions, cultured normal pigment epithelium continues to phagocytize large amounts of normal (Fig. 2d) or RCS outer segment fragments.

The reduced ability of cultured RCS pigment epithelium to phagocytize outer segment material establishes that the RCS mutant gene is expressed as a defect in the phagocytic mechanism in pigment epithelial cells. This is not a general defect in phagocytosis, since RCS pigment epithelium is capable of ingesting polystyrene spheres in vitro. The envelopment of outer segments by microvillous processes of RCS pigment epithelial cells indicates that the RCS cells respond to the presence of outer segments, a phenomenon also observed in the RCS rat in vivo (*5*). The fact that RCS rat outer segment fragments are phagocytized by cultured normal rat pigment epithelium is direct evidence that the RCS mutant gene is not expressed in photoreceptor cells in a way that prevents phagocytosis of RCS outer segment material. This in vitro experimental system now makes accessible to further study the genetic defect in the phagocytic mechanism of the pigment epithelial cell which is responsible for photoreceptor cell degeneration in the RCS rat.

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12. Polystyrene (Latex) spheres (Dow Chemical) were rinsed three times in distilled water by centrifugation and resuspended to 0.04 percent (weight to volume) in culture medium, and 100-μl portions of the suspension were added to pigment epithelium explants.
13. Cultures were fixed with 2 percent osmium tetroxide in 0.2*M* sodium cacodylate buffer (*p*H 7.35) at 0°C for 30 minutes. The cultures were then rinsed with cold distilled water and dehydrated in a graded series of ethanol solutions. The explants were loosened from the plastic surface with propylene oxide and embedded in Epon 812. Thin sections cut perpendicular to the culture substratum were stained with uranyl acetate and lead citrate.
14. We thank E. F. McCarthy, Jr., for technical assistance. Supported by grants from the National Retinitis Pigmentosa Foundation, Baltimore, Md., and the George Gund Foundation, Cleveland, Ohio. Breeding pairs of RCS-p^+ rats were provided through National Institutes of Health research contract EY-3-2133 to R. L. Sidman.

13 January 1977; revised 4 March 1977

Conformations of Prostaglandin $F_{2\alpha}$ and Recognition of Prostaglandins by Their Receptors

Abstract. *The conformation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been determined by x-ray diffraction techniques. Two independent conformers of $PGF_{2\alpha}$, studied as the tris(hydroxymethyl)methylamine salt, are observed to adopt the familiar "hairpin" conformation with the α and ω chains aligned roughly parallel. The conformers differ in ring conformation and at the C(17)–C(18) bond, one adopting a C(9) envelope ring conformation and a* trans *geometry at the C(17)–C(18) bond, while the other adopts a C(8) envelope ring conformation and a novel* gauche *geometry about C(17)–C(18). Comparison of the conformation of $PGF_{2\alpha}$ with that of prostaglandin E_2 suggests a recognition mechanism which would permit $PGF_{2\alpha}$ and prostaglandin E receptors to distinguish between the two potent prostaglandins. The recognition model explains much of the binding data for the $PGF_{2\alpha}$ receptor in the corpus luteum and predicts the existence of an interesting $PGF_{2\alpha}$ analog.*

By virtue of its diversity of action and potential for pharmacological exploitation, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is perhaps the most interesting of the classical prostaglandins. It plays an important role in luteolysis and is clinically employed in pregnancy termination and induction of labor at term. Prostaglandin $F_{2\alpha}$ and prostaglandin E_2 (PGE_2) are the most abundant classical prostaglandins. Recent binding studies (*1*) reveal that $PGF_{2\alpha}$ and PGE_2 have little affinity for each other's receptors, suggesting that the conformations of the two prostaglandins differ to a significant degree, even though they differ chemically only in the saturation of the C(9)–O(9) bond (Fig. 1). Having previously determined the conformation of PGE_2 by diffraction techniques (*2*), we now report the determination of the molecular conformations of $PGF_{2\alpha}$, and offer plausible explanations for the recognition of prostaglandins by their receptors.

Prostaglandin $F_{2\alpha}$ was crystallized as its tris(hydroxymethyl)methylamine (tris) salt from a methanol-acetonitrile solution. The crystals are triclinic, space group $P\bar{1}$, with unit cell axial lengths $a = 8.330(3)$, $b = 26.458(6)$, and $c = 6.221(2)$ Å; angles $\alpha = 99.98(3)°$, $\beta = 91.75(4)°$, and $\gamma = 97.88(3)°$; and volume 1335.6 Å^3. The observed density, 1.189 g/cm^3, measured by flotation in a chloroform-benzene mixture, implies that there are two independent molecules of $PGF_{2\alpha}$-tris salt per asymmetric unit (calculated density, 1.183 g/cm^3). Of 5472 independent reflections, measured in the θ-2θ scan mode on an automatic diffractometer employing Cu$K\bar{\alpha}$ radiation, 4820 are considered to be observed (that is, their intensities are more than twice their standard deviations). This is an unusually favorable percentage of observed data for prostaglandin crystals, reflecting the quality of the data. The structure was determined by application of a system of programs, QTAN, written by one of us (D.A.L.) and described previously (*3*). The model has been refined by using anisotropic thermal parameters for carbon, nitrogen, and oxygen atoms to a current residual $R = 0.08$.

The two conformers of $PGF_{2\alpha}$ are shown in Fig. 2. Although distinct in various ways, both display the "hairpin" conformation observed for other active prostaglandins (*4*); that is, the α [C(1) through C(7)] and ω [C(13) through C(20)] side chains of each conformer align in such a way that they run roughly parallel. To accomplish this, the ω chains twist (*5*) at the C(15) positions [torsion angles C(13)–C(14)–C(15)–C(16) are −150° and −118° for conformers A and B, respectively], effectively positioning the O(15) hydroxyl oxygens out and away from the centroids of the conformers. In a description of L-shaped prostaglandin B_1, DeTitta (*6*) discussed the importance of the hairpin conformation and the particular importance of the position of the C(15) hydroxyl with respect to the metabolizing enzyme 15-