ence between the average phase of the deprived and nondeprived eyes corresponds to a difference in latency of about 15 msec at 0.12 c/deg. All other effects were not statistically significant.

Discussion. The VEP records indicate that the long periods of monocular lid suture produced a severe functional amblyopia in the deprived eye of each cat. The attenuation of the VEP produced during stimulation of the deprived eye was larger than that reported in earlier studies, and was due probably to a longer period of deprivation (3–4 yr of deprivation in the current study vs 9–12 months of deprivation in earlier studies).

Although 3–4 yr of monocular lid suture produced a formidable reduction in the amplitude of the VEP, there were no consistent effects of the monocular lid suture on the FERG and PERG. FERGs were nearly identical for the deprived and nondeprived eyes and comparable to those obtained in normal animals. Some changes in PERGs were observed for the deprived eye, but those changes were small and inconsistent relative to the pervasive effects of monocular lid suture on the cortical VEP.*

The current results are consistent with earlier observations that deficits in the dorsal lateral geniculate nucleus and in the visual cortex occur after rearing with monocular lid suture, whereas retinal ganglion cells in the deprived eye seem to function normally. Moreover, the current data indicate that, even after long periods of monocular lid suture, the cat’s retina remains relatively immune to the deleterious effects of visual deprivation. This pattern of results in cats suggests that the PERG, as an electrodiagnostic tool, would not delineate the severity of an amblyopia.

Key words: electroretinogram, visual evoked potential, visual deprivation, cat

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Serial Adoptive Transfer of Experimental Autoimmune Uveoretinitis In Rats

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Experimental autoimmune uveoretinitis (EAU) and pinealitis induced by an interphotoreceptor retinoid-binding protein (IRBP)-derived peptide (R4) was serially transferred into naive recipient rats, using spleen cells from recipients of previous “orders” of transfer. The cells initiating the disease in recipients of the first order were either lymph node cells from rats immunized against peptide R4, or lymphocytes of a cell line specific toward this peptide. The serial transfer was successfully carried out through as many as four orders of sequential recipients. Invest Ophthalmol Vis Sci 31:1409-1412, 1990

Experimental autoimmune uveoretinitis (EAU) is an ocular inflammatory disease that is induced in
animals by immunization with ocular-specific antigens. EAU resembles certain uveitic conditions in humans and is considered a model for these conditions. The two retinal proteins used most often for EAU induction are S-antigen and interphotoreceptor retinoid-binding protein (IRBP). In addition, synthetic peptides derived from these retinal proteins have also been shown to be uveitogenic. S-antigen and IRBP also localize in the pineal gland, and consequently, animals immunized with these proteins usually develop pinealitis (“experimental autoimmune pinealitis,” or EAP) as well. We and other investigators have shown that EAU can be adoptively transferred into naive recipients by injection of lymphocytes sensitized against the retinal antigens. Furthermore, EAU and EAP can also be adoptively transferred by cell line lymphocytes specific toward the retinal antigens or their peptides.

We report here that EAU and EAP can be serially transferred into naive rats, using spleen cells (SpC) from recipients of previous “orders” of serial transfers. The disease in these experiments was initiated in the first order of recipients by lymph node cells (LNC) or line cells sensitized against an IRBP-derived peptide, designated R4, which comprises residues 1158-1180 of bovine IRBP.

Materials and Methods. Antigen: The IRBP-derived peptide R4 (sequence 1158-1180 of bovine IRBP: HVDDTDLYLTPTARSVGAADGS) was synthesized and purified by Applied Biosystems (Foster City, CA) with the t-BOC chemistry, on a 430A peptide synthesizer (Applied Biosystems).

Animals: Male inbred Lewis rats, 8-12 weeks old, were supplied by Charles River (Raleigh, NC). Treatment of animals was in compliance with the ARVO Resolution on the Use of Animals in Research.

Cells used for initiation of EAU in recipients of the first order: Peptide R4-sensitized lymphocytes of two sources were used in this study, as follows. 1) Draining LNC from rats immunized with R4 (200 μg/rat), as described elsewhere: The LNC were collected 14 days after immunization and cultured with the peptide (at 15 μg/ml) for 3 days, as detailed in another communication, before being injected intraperitoneally (IP) into the recipient rats. 2) Lymphocytes from a cell line (designated R4G), which was established as described elsewhere: The line cells were used after three cycles of alternating stimulation with R4 and interleukin 2 and were stimulated with R4 (at 10 μg/ml) for 3 days before being injected (IP) into the recipients.

Serial adoptive transfer of EAU: All recipient rats were killed 5 days after cell transfer and their SpC were cultured with peptide R4, at 15 μg/ml, using the procedure described elsewhere. After incubation for 3 days, the cells were washed thoroughly and injected IP into naive recipient rats of the subsequent order. Donor rat spleens yielded 4–6 × 10^8 lymphoid cells, and 30–50% of these cells were recovered after the incubation with the peptide.

Disease monitoring and assessment: EAU development was monitored by clinical examination, while the severity of the ocular inflammatory changes was evaluated by both clinical and histologic examinations, using a scale of 0–4. Pineal changes were assessed by histologic examination.

Results. Table 1 summarizes the data of two experiments in which EAU was serially transferred into naive recipients. The cells initiating disease in the recipients of the first order were R4-stimulated LNC from donors actively immunized with this peptide (experiment 1), or lymphocytes of cell line R4G, which is specific toward R4 (experiment 2). In both experiments, the serial transfer of EAU was carried out with the SpC of recipients of the previous order, after stimulation in culture with peptide R4. The exp-

<table>
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<th>Experimen</th>
<th>Recipient order</th>
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<th>Number (×10^-6)</th>
<th>Onset day</th>
<th>Severity</th>
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<td>1</td>
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* Days after cell injection.
† Severity of disease, on a scale of 0-4, was evaluated by clinical and histologic examinations.
‡ Disease detected only by histologic examination.

LNC, lymph node cells; SpC, spleen cells.
Fig. 1. Inflammatory changes in recipient rats of serially transferred disease. The disease was initiated by peptide R4-sensitized LNC (Experiment 1 of Table 1). (a) Posterior segment of an eye of a recipient of the second order of serial transfer. Inflammatory cells infiltrated the retina and caused a focal destruction of the photoreceptor cell layer. Inflammatory cells also are seen in the subretinal space, in particular beneath the folding of the photoreceptor cell layer. Many inflammatory cells accumulated in the thickened choroid (hematoxylin and eosin, ×20). (b) Posterior segment of an eye of a recipient rat of the third order. Accumulation of inflammatory cells is seen in the vitreous and retina and at the optic nerve head. The choroid is thickened and infiltrated with inflammatory cells (hematoxylin and eosin, ×120). (c) Pinalitis in the recipient rat of the third order. Scattered foci of inflammation (arrows) are seen throughout the pineal gland (hematoxylin and eosin, ×30). Inset: Higher magnification of an inflammatory focus (hematoxylin and eosin, ×120).

Experimental procedure was found to be similarly successful with the two initiating cell populations; recipients of three orders of serial transfer developed both clinical and histologic changes that were detected 4–5 days after cell injection. A recipient of the fourth order of serial transfer developed mild disease which was detected only by histologic examination (Experiment 1). The severity of inflammatory changes was found to decrease gradually in recipients of the higher orders of serial transfer.

Figure 1 shows typical histologic changes in eyes of recipients of the second and third orders of serial transfer. The ocular changes in the rat of the second order (Fig. 1a) closely resemble those seen in rats actively immunized with peptide R4, whereas the recipient rat of the third order developed milder inflammatory changes, mainly at the optic nerve head (Fig. 1b) and in the anterior segment of the eye (data not shown). In addition to the ocular changes, the recipient rats developed pineal changes that typically consisted of inflammatory foci scattered throughout the gland (Fig. 1c).

Discussion. Data reported here show for the first time that EAU and EAP can be serially transferred as many as four times, using the SpC from recipients of previous orders of serial transfer. This finding is in accord with data reported by Holda et al and by Wegmann and Hinrichs, showing serial transfer of another autoimmune disease, experimental allergic encephalomyelitis (EAE).

In the current study, all cell preparations were injected after incubation with the specific epitope, peptide R4, and it could be argued that EAU was induced in recipient rats by the residual peptide that was carried over with the injected cells. This possibility seems unlikely, however, in view of the following. 1) The onset time of EAU in the recipients, 4 or 5 days after cell injection, is much shorter than the onset time of disease in rats actively immunized with this peptide (12 days). 2) In other experiments, not recorded here, the cell line lymphocytes were found to be capable of transferring EAU after stimulation in vitro with a polyclonal mitogen, concanavalin A. 3) As shown by Wegmann and Hinrichs, the serial transfer of EAF was mediated solely by the injected cell line lymphocytes and at the optic nerve head. The choroid is thickened and infiltrated with inflammatory cells (hematoxylin and eosin, ×120). (c) Pinalitis in the recipient rat of the third order. Scattered foci of inflammation (arrows) are seen throughout the pineal gland (hematoxylin and eosin, ×30). Inset: Higher magnification of an inflammatory focus (hematoxylin and eosin, ×120).
cells with no significant involvement of the recipients' lymphocytes.

The finding that SpC from recipient rats of three orders of serial transfer were capable of inducing EAU in naive rats suggests that a large proportion of the specifically sensitized lymphocytes home to the spleen of the injected recipients. Furthermore, since the injected cells are diluted out by the naive recipients' own splenocytes, it is conceivable that the injected cells proliferate vigorously in these recipients, probably in response to the prior stimulation in culture.

Another noteworthy aspect of the current study concerns the uveitogenic molecule used here, peptide R4. This peptide was found in other studies to be a nonimmunodominant determinant of IRBP and to exhibit a low level of uveitogenic capacity, as expressed by the high dose of R4 needed to induce disease. It is remarkable, therefore, that lymphocytes sensitized against peptide R4 exhibited the capacity to serially transfer the disease through as many as four orders of recipients. The latter finding is consistent with our previous observation that lymphocytes sensitized toward R4 exhibit high capacity to adoptively transfer EAU. In fact, lymphocytes sensitized against R4 resemble cells sensitized against an immunodominant and highly uveitogenic peptide (R14) in their capacity to adoptively transfer disease. These observations thus support our idea that the low uveitogenicity of peptide R4 is attributable mainly to its low capacity to sensitize T-lymphocytes.

Key words: experimental autoimmune uveoretinitis (EAU), experimental autoimmune pinealitis (EAP), serial adoptive transfer of disease, interphotoreceptor retinoid-binding protein (IRBP), IRBP-derived peptides

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