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# Macrophage Subpopulations and RPE Elimination in the Pathogenesis of Experimental Autoimmune Pigment Epithelial Protein-Induced Uveitis (EAPU)

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Experimental autoimmune pigment epithelial protein-induced uveitis (EAPU) is a new type of disease that destroys the retinal pigment epithelium (RPE), and exhibits a hitherto unknown form of progressive chorioretinal dystrophy in which neuroretinal inflammatory foci are absent. The present study was aimed at studying the expression of adhesion molecules, and the kinetics of the appearance of the main types of macrophages and other intraocular immunocompetent cell populations in the various stages of this disease. EAPU was evoked in Lewis rats by immunization with the membrane protein from bovine RPE cells containing PEP-65 as main constituent. In the uvea, increased expression of intercellular adhesion molecule-1, of class II major histocompatibility complex antigen, and of ED2 macrophage reactivity were observed closely before the onset of EAPU. Expression of these reactivities was also slightly elevated by injections of the applied adjuvants alone. The onset of EAPU was mainly characterized by initial uveal infiltrations of ED1+ macrophages and a minor population of CD4 T cells, and an increase in ED3, ED7 and perivascular ED2 reactive macrophages. This was followed by the development of focal accumulations of ED1+ cells at both sides of the Bruch's membrane–RPE layer (Dälén–Fuchs nodules) which was permeated and disintegrated at these sites. The outer choroidal layer, the anterior iridal surface, and the base of the ciliary body more frequently contained active inflammatory cells than the other uveal areas. Lymphoid cells were found scattered through the uvea, aqueous and vitreous. The sites of increased activity of ED2+ and ED3+ cells in the uvea were rather similar to those of ED1 macrophages in the various stages of EAPU. Starting from multiple foci, the process of the formation of plaque-shaped cell accumulations in severe EAPU progressed along the RPE and exhibited a chronic character. The results of this study show that ED1+, ED2+, ED3+ and ED7+ subpopulations of macrophages are actively involved in an immunopathological process in which the RPE is the target. The thickening of the plaque-shaped cell accumulations stops if the integrity of all RPE cells at that site has been affected. We postulate that this is the result of antigen elimination while additional influence of the abrogation of RPE cytokine production is presumed. © 1996 Academic Press Limited

*Key words:* experimental autoimmune pigment epithelial protein-induced uveitis (EAPU); immunocytochemistry; macrophages subpopulations; retinal pigment epithelium; PEP-65.

## 1. Introduction

The autoantigenic and pathogenic nature of several retinal photoreceptor proteins has been studied in detail. They evoke experimental autoimmune uveoretinitis (EAU), an inflammatory process that includes retinitis with elimination of the photoreceptors, and pinealitis as main features (Gery, Mochizuki and Nussenblatt, 1986). Recently it has been shown that in addition to the photoreceptor cells, also the cells of the retinal pigment epithelium (RPE) contain intrinsic autoantigens with a potential pathogenicity. Purified RPE, choroidal or iris melanin injected in adjuvant with pertussis toxin as coadjuvant appear to be uveitogenic even at the level of 1 µg melanin-protein (Broekhuysse, Kuhlmann and Winkens, 1992a, 1993). In this form of experimental melanin-protein-induced uveitis (EMIU), retinitis is absent. EMIU (also called

EAAU, experimental autoimmune anterior uveitis) exhibits recurrences (Chan et al., 1994; Broekhuysse et al., 1995a), and intraperitoneal injection of melanin-protein suffices to evoke the disease (Broekhuysse et al., 1995b).

It was recently found that both the buffer-insoluble, Triton X-100-soluble fraction of RPE cells, and its main 65 kDa pigment epithelial polypeptide PEP-65 are capable of inducing experimental autoimmune pigment epithelial protein-induced uveitis (EAPU) (Broekhuysse, Kuhlmann and Winkens, 1992b). The histopathological pattern of onset and progression of this disease differ markedly from that of photoreceptor cell antigen-induced forms of EAU, and from EMIU. EAPU exhibits progressively extending, epitheloid cell accumulations along the RPE-Bruch's membrane layer which is destroyed while inflammatory foci within the neuroretina are virtually absent. The present immunocytochemical study was designed to identify the main immunocompetent cells involved in the pathogenesis of EAPU.

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## 2. Materials and Methods

### Antigen

Bovine eyes were transported on ice to the laboratory within 3 hr after enucleation. The eyes were dissected and the RPE cells and the RPE antigen were isolated as described previously (Broekhuysse et al., 1992a, 1992b). Briefly, the RPE cells were freed from rod outer segments and other tissue fragments by five to seven sedimentations from buffered sucrose (0.25M; 15 mM phosphate buffer; pH 7.3) at 140 *g* (10 min, 20°C). The elimination of the rod outer segments was monitored by phase contrast microscopy. This careful purification procedure was necessary in order to obtain a final antigen preparation in which opsin was virtually absent. The RPE cells were extracted by phosphate buffered physiological saline pH 7.4 (PBS), and the insoluble membranous residue was extracted by Triton X-100. The supernatants containing the Triton X-100 soluble proteins of the RPE (RPE-TS) with its main constituent PEP-65 were stored at -25°C. The determination of protein, and the check for purity of the antigen by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting have been described previously (Broekhuysse et al., 1991a, 1992a, 1992b).

### Animal Experiments

Groups of six female Lewis rats (150–180 g) for each examined stage of EAPU were immunized with 150  $\mu$ g RPE-TS. Half the antigen dose was emulsified in Freund's complete adjuvant (CFA; Difco Laboratories, Detroit, MI, U.S.A.) and injected in the hind foot

pads (0.1 ml per pad). The other half was mixed with 1 ml PBS containing 2  $\mu$ g pertussis toxin (Sigma Chemical Company, St. Louis, MO, U.S.A.) and injected intraperitoneally. Starting from day 8 after immunization, the eyes were examined daily for 2 weeks and subsequently twice a week. Clinical signs of uveitis were monitored by direct slit-lamp biomicroscopy after dilation of the pupils by tropicamide (0.17%). The observations were graded on a scale of 0–4 as described previously (Broekhuysse et al., 1992b). The experiments were terminated at different time points (at days 4, 7, 8, 9, 10, 12, 14 and 60 after immunization) in order to study pre-stages, onset, mid-stage (peak of inflammation) and a late stage of the development of EAPU as described in Results. Control rats were injected as described above with CFA/PBS emulsions (without antigen) with or without pertussis toxin, or received only pertussis toxin (each group three rats), or no injections at all (six rats). The left eyes of EAPU rats were fixed in Bouin solution, and processed according to standard histological techniques. The right eyes, and both eyes of controls were prepared for immunocytochemistry as described below. Four eyes of the severe stage (day 12) were processed for electron microscopy (Broekhuysse et al., 1991b). All eyes were sectioned parallel to the optic axis.

### Immunocytochemistry

Enucleated eyes were snap frozen in optimal cutting temperature compound (Miles Laboratories, Naperville, IL, U.S.A.). Sections of 7  $\mu$ m were placed on poly-L-lysine coated slides, dried for 30 min in a cold air stream, and stored at -80°C until use. For immunostaining, sections were fixed in acetone

TABLE I  
*Specificity of monoclonal antibodies*

Antibody	Specificity	Reference
ED1	Lysosomal antigen in rat monocytes, macrophages, and most dendritic cells	Damoiseaux et al., 1994
ED2	Resident macrophage (membrane) antigen	Dijkstra et al., 1985
ED3	Activated macrophages	Van den Berg et al., 1992
ED7	CD11b. Adherent macrophages, granulocytes, dendritic cells, NK	Huitinga et al., 1993
OX-6	MHC class II antigen (Ia equivalent) on dendritic cells, activated T cells, and macrophages	McMaster and Williams, 1979
OX-8	CD8 antigen. MHC class I restricted T lymphocytes, NK cells	Barclay, 1981
W3/25	CD4 T cells, some macrophages, and dendritic cells	Jefferies, Green and Williams, 1985
R73	TcR $\alpha/\beta$ molecule on (peripheral) T cells	Hünig et al., 1989
W3/13HLK	CD43 antigen. T cells, plasma cells, neutrophils	Brown et al., 1981
1A29	CD54. Intercellular adhesion molecule ICAM-1 on activated endothelium	Tamatani and Miyasaka, 1990
RCK102	Cytokeratins 5 + 8	Ramaekers et al., 1987
M3F7	Collagen IV	Ishizaki et al., 1993



(5 min, 4°C), washed in PBS, and incubated with monoclonal antibodies for 60 min in a moist chamber. The sections were then washed three times for 5 min in PBS, and incubated for 60 min with horseradish peroxidase-conjugated rabbit anti-mouse Ig (Dakopatts, Glostrup, Denmark) (Kuijpers et al., 1991), using diaminobenzidine (in case of 1A29) or aminoethylcarbazole (all other antibodies) as substrate.

The following mouse monoclonal antibodies were used (Table I): OX-6, OX-8, W3/25 and W3/13HLK (Seralab, U.K.), 1A29 and R73 (Serotec, Indianapolis, IN, U.S.A.) ED1, ED2, ED3 and ED7 (Dr C. D. Dijkstra, Amsterdam, The Netherlands). M3F7 (Developmental Studies Hybridoma Bank, Baltimore, MD, U.S.A.), and RCK102 (Dr F. C. S. Ramaekers, Maastricht, The Netherlands) were used for additional identification or localization of cells and tissues. Mouse IgG (Serotec) was the control primary antibody.

### 3. Results

#### *Clinical Features of EAPU*

The first signs of EAPU consisted of cells and protein aggregates in the anterior chamber at day 9 after immunization. Transient flare and cells peaked sharply at day 10. In severe reactions it lasted several days before the fundal view became (virtually) clear. Marked, often peripherally situated, irregular shaped areas of vitreous cell infiltrations were observed which remained usually 1 or 2 weeks after onset. During this period a short-lasting retinal protrusion was noted in some rats with severe inflammation. In a few cases, scarce vitreous cell aggregates were still observed in the late stage at day 60.

#### *Histopathology of EAPU Development*

Starting from day 9 after immunization signs of multifocal choroiditis could histologically be observed. This stage could become stationary in mild disease. In most cases severe EAPU developed as a form of panuveitis, and epitheloid cells accumulated focally along both sides of the Bruch's membrane-RPE layer and in the ciliary body during the next days. Along the RPE, they initially resembled Dálen-Fuchs noduli. Inflammatory foci were frequently localized in the chorioretinal periphery and ciliary body. Depending on severity, some of the cell accumulations extended progressively along the RPE during the next months, and lifted the neural retina. The photoreceptor layer remained largely intact but was finally damaged along these cell plaques without clear signs of inflammation of the neural retina. No phagocytosis by macrophages was noted at the light microscopic level. Pinealitis was absent.

#### *Immunocytochemistry*

ICAM-1 expression on endothelial cells was spor-

adically present in the uvea and conjunctiva of the four types of controls described in section 2, and of rats at day 4 and 7 after immunization. A slight increase was noticed at day 8 in the iris, one day before flare and cells entered the anterior chamber. From that day ICAM-1 expression was increasingly present in the uvea, especially at the endothelium of the choroid [Fig. 1(A)]. This reactivity remained at least until the late stage (day 60). No expression was detected in retinal vessels. Constitutional class II antigen expression was occasionally seen on uveal cells of untreated animals. This was slightly increased by injection of either pertussis toxin or CFA, or both, and at day 4 after immunization. It occurred especially at the base of the ciliary stroma and in the outer choroidal layer where cell infiltration started a few days later. The stained cells included uveal dendritiform cells. The choroidal reactivity increased at day 7 and 8, the anterior uveal reactivity at day 9–14. MHC class II antigen expression was predominantly found on cells in inflammatory foci. In the late stage it decreased to variable, usual moderate levels.

Staining with R73, OX-8, W3/13HLK and W3/25 monoclonal antibodies showed that small numbers of T non-helper cells (mostly CD8; R73+/OX8+/W3/25-) and more T helper cells (R73+/OX8-/W3/25+) were present in various ocular tissues from day 9 after immunization. The cells were found in cell accumulations as well as scattered through the uvea (especially the choroid), the aqueous humor, anterior and posterior vitreous, along the corneal endothelium and in small vessels along the interface of choroid and sclera, but they were absent in cornea and sclera. In severe inflammation, scattered cells were also seen in the neural retina. During the development of EAPU, OX-6 increasingly stained dendritiform cells (Langerhans cells; Kusuda et al., 1989) in peripheral sclera and conjunctiva. Staining of these cells in corneal limbus was similar before and during inflammation. There was little change in the T cell distribution pattern during the various stages but T helper cell infiltration increased towards later stages. T cells constituted a minor population in the cell accumulations. Virtually no plasma cells and polymorphonuclear leukocytes were found according to morphological criteria and scarce reactivity with W3/13 and ED7, respectively.

#### *Immunocytochemistry of Intraocular Macrophages*

*Normal eye.* Except the class II antigen expressing cells which were described above, ED2+ uveal resident tissue macrophages were identified as well. They were frequently located near blood vessels, and were often seen at the anterior iris surface, at the base of the ciliary stroma, in the conjunctiva, and in the outer layer of the choroid, and between episclera and extraocular tissue. Identification of small vessels was facilitated by the reactivity of the basement membrane



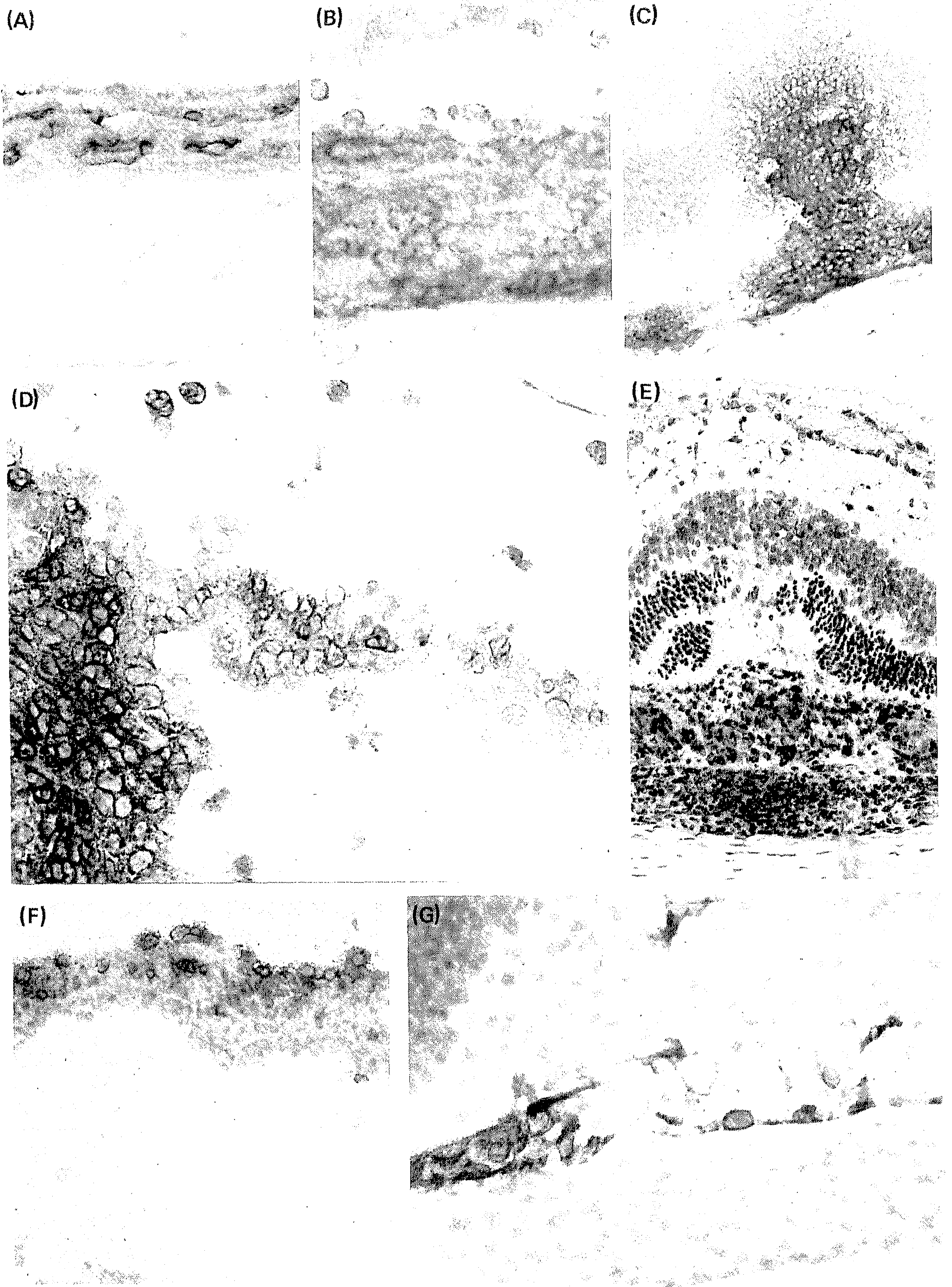


FIG. 1. Several stages in the development of severe EAPU. (A) Day 8 after immunization. Endothelial ICAM-1 presentation in the choroid just before or at the onset of uveitis ( $\times 400$ ). (B) ED1 + cell accumulation in choroiditis (day 9). Some macrophages are present within the RPE-Bruch's membrane layer, and also subretinally ( $\times 400$ ). (C) Severe choroiditis at day 10. OX-6 staining. Macrophage accumulation in the choroid in a focus extending through the RPE into the photoreceptor layer. Macrophages are virtually absent in the neural retina but scattered lymphoid cells are present. Note that the RPE is OX-6 negative ( $\times 200$ ). (D) Anterior uveitis at day 12. The ciliary stroma is filled with (ED7 +) macrophages whereas the iris is mildly infiltrated ( $\times 400$ ). (E) Day 12, ED1 staining. Cell accumulations containing many macrophages at both sides of the RPE layer. Scattered inflammatory cells in the neural retina which is distorted. The photoreceptor layer is focally disrupted ( $\times 200$ ).



TABLE II  
Summary of immunostaining of uveal tissues and cells

EAPU stage	Days After immunization	Uveal tissue*	Cell marker†								
			1A29	OX-6	W3/25	OX-8	R73	ED1	ED2	ED3	ED7
No injections	—	A	0	±	±	0	0	±	+	+	±
		P	±	±	±	0	0	±	+	+	±
Pre-stage	4	A	±	+	±	0	0	±	+	+	±
		P	±	+	±	+	0	±	++	+	±
Pre-stage	7	A	±	+	±	0	0	±	+	±	±
		P	±	++	±	+	0	+	++	+	±
Pre-stage	8	A	+	±	±	0	0	+	+	+	+
		P	++	++	+	+	+	+	+++	+	+
Early stage	9	A	++	+++	+	++	±	++	++	++	++
		P	++	++	+	±	+	+++	+++	+	+++
Peak stage	10-14	A	++	+++	+++	++	++	+++	+++	+++	+++
		P	+++	[+++]	+++	+	[++]	++++	++	++	+++
Late stage	60	A	+	+++	+++	±	±	++	+	+	+
		P	+	++	++	+	[++]	[+++]	++	++	+++

\* A, Anterior uvea (iris, ciliary body); P, posterior uvea.

† Immunostaining is graded from 0 to + + + +, and is based on the relative intensity of staining and the number of cells. ±, sporadic reactivity or reactive cells, 1-5 cells per tissue section; +, 5-15 cells; ++, 15-30 cells; + + +, tissue moderately filled with reactive cells; + + + +, very dense cell population, mostly accumulations. Staining predominantly within cell accumulations is indicated between brackets.

collagen IV with M3F7 antibody. The scarce sites with ED3 staining in normal eyes closely corresponded to ED2 reactivity. Occasional ED1 + cells were observed within the blood vessels. The same patterns of ED reactivities were observed in adjuvant-injected animals. A summary of the main results for normal eyes and for the various stages of EAPU is given in Table II.

*First signs of inflammation (days 4-8).* Already at days 4 and 7 after immunization a slight increase in uveal ED2 reactivity was noticeable which became clearly manifest at day 8 just before the onset of EAPU (Table II). ED3 stained the same sites. At day 7, ED1 + and ED7 + cells were mainly seen within vessels in the outer layer of the choroid, and scarcely within the choroid. These cells and ED2 + cells were also present in the base of the ciliary stroma and sporadically in the adjacent sclera and in the iris, in or adjacent to blood vessels. Some uveal ED7 + macrophages were seen at day 8.

*Early stage of EAPU (day 9).* ED1 + macrophage infiltration in the choroid was markedly increased. Close to extended cell accumulations, ED1 + cells were sometimes seen within the RPE layer, and subretinally [Fig. 1(B)]. The ciliary stroma stained extensively at its base, and some ED1 + cells were also seen in the surrounding ocular chamber. The numerous ED1 + cells appearing in the uveal tissues showed irregular

cytoplasmic ED1 reactivity characteristic for macrophages (Dijkstra et al., 1985). Increasing numbers of these cells were present in the anterior layer of the iris and in the vitreous body. The neural retina remained unaffected. The increases in ED2 + and ED7 + cells (and to a lesser extent in ED3 + cells) were similar to those of ED1 + cells in the tissues.

*Severe inflammation (days 10-14).* At this stage, the subretinal space became rapidly infiltrated by ED1 + and ED7 + cells which seemed to penetrate this area from previously formed choroidal cell accumulations [Fig. 1(B) and (C)]. No class II antigen expression was observed at the RPE [Fig. 1(C)] or at other intraocular epithelia in any of the stages of EAPU. Extensive inflamed areas were frequently found at the chorioretinal periphery where the ciliary body became affected as well. The cell collections contained ED1 + and ED7 + macrophages [Fig. 1(D)] while lymphocytes (R73 +) constituted a minority (Table II). A moderately weak W3/25-positive macrophage population was found in the uveal ED1 + macrophage accumulations. Once formed, the collections of phagocytes appeared immobile at these sites. In several cases of very severe EAPU, the photoreceptor layer became fully disorganized and disrupted [Fig. 1(E)] without the presence of neuroretinitis. In addition, ED1 + /ED2 - cells were noticed in the inner retinal layers between the inner limiting membrane and the outer nuclear

(F) ED3 + cells in the anterior iridal layer at day 12 (× 200). (G) Choroidal-subretinal cell accumulation at day 14. RCK102 (cytokeratin 5 + 8) staining shows a disintegrating RPE. Macrophages are present between the layers of Bruch's membrane (× 400).



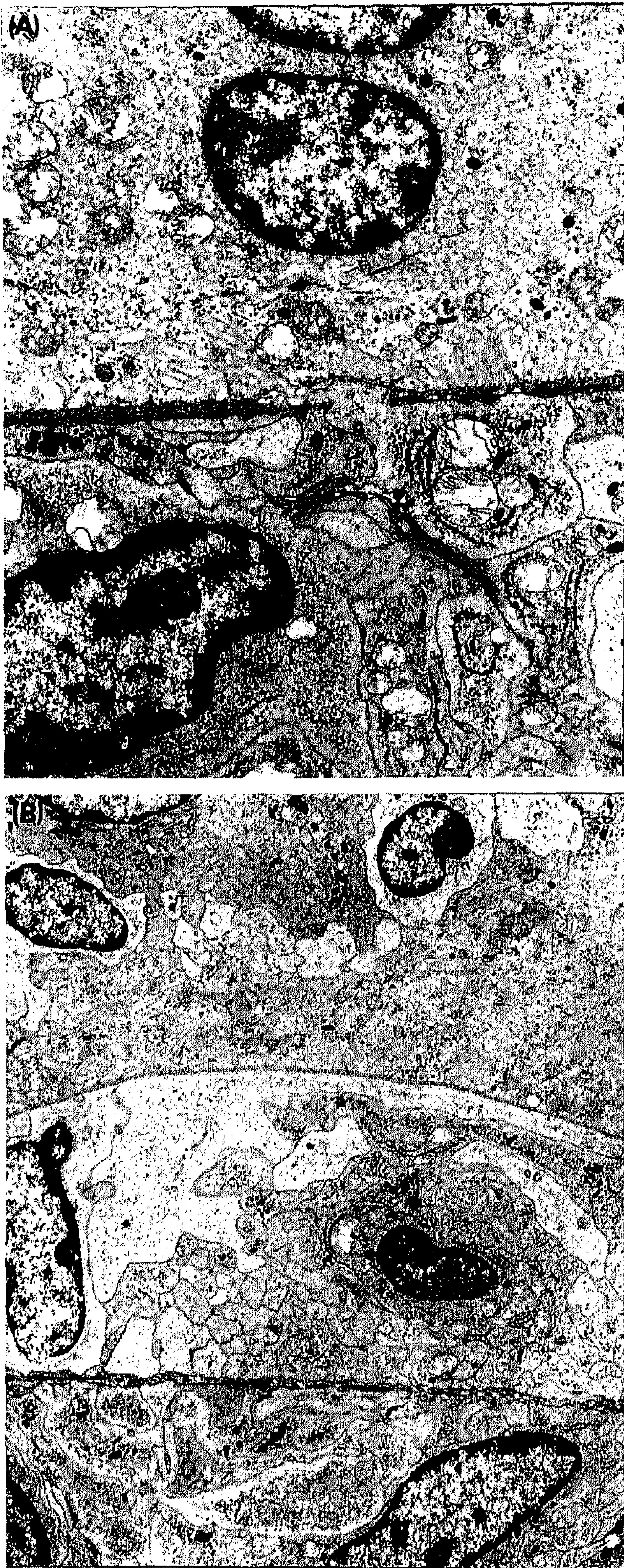


FIG. 2. Severe EAPU (day 12). Ultrastructural detail of a Dalen-Fuchs nodule. (A) A choroidal macrophage has disrupted Bruch's membrane, and protrudes into the RPE (upper part of the picture) ( $\times 4200$ ). (B) A macrophage between the dissociated layers of Bruch's membrane ( $\times 8500$ ).

layer near inflammatory foci [Fig. 1(E)]. These cells represented locally attracted monocytes in retinal capillaries (localized by M3F7 staining), and activated dendritiform cells in the inner nuclear layer. They were not found in unaffected retinal areas or in control rat retinas. Scattered ED2+ or ED3+ cells were present in the choroid, the vitreous, at the anterior iridal

surface [Fig. 1(F)], occasionally in the ciliary body, and in subretinal fluid in case of retinal detachment. Choroidal-subretinal cell accumulations were moderately reactive for ED2 and ED3 (Table II). Resident macrophages (ED2+) were also found in adherent retrobulbar tissue and in considerable numbers in conjunctiva. The vitreous body was infiltrated by a cell population of similar composition.

A closer examination of the blood-retina barrier by application of the RCK102 antibody to cytokeratin 5+8 showed that it was reactive with the RPE and the inner epithelium of the ciliary body in normal as well as in inflamed eyes. Within cell accumulations, the RPE-Bruch's membrane layer appeared to be focally absent or exhibited partially a foamy or multi-membrane appearance [Fig. 1(G)]. Separated membrane remnants were occasionally seen in the subretinal part of the nodules. Electron microscopic pictures showed phagocytes permeating Bruch's membrane [Fig. 2(A)], between the dissociated layers [Fig. 2(B)], and disturbing RPE cell integrity.

*Late stage of EAPU (day 60).* Extended ED1+ macrophage accumulations especially in the posterior uvea, and in the adjacent subretinal space were characteristic for the late stage of EAPU. They contained ED2, as well as ED3 or ED7 positive cells and remnants of cells. Twenty to fifty per cent of the RPE was included and infiltrated by these cell collections, and had been destroyed. At the same time small macrophage accumulations were also present along the RPE. Scattered ED1+ and ED2+ cells were observed in the anterior uvea (especially at the iridal anterior surface), while an occasional nodule around a vessel was found which contained many ED2+ cells. Scarce ED2+ cells were present in the trabeculum and the anterior chamber.

If the primary monoclonal antibody was replaced by mouse IgG in the staining procedures the sections remained always unstained.

#### 4. Discussion

##### *Development of EAPU*

EAPU is a form of experimental ocular inflammation in the Lewis rat that clinically exhibits anterior uveitis during its onset, but histopathologically represents chronic panuveitis. In most photoreceptor antigen-induced forms of EAU in the rat a similar overall pattern is observed (Gery et al., 1986), but retinitis and pinealitis than become main features, and the photoreceptors are the target. The hallmark of EAPU is the development of Dalen-Fuchs noduli into large plaque-shaped cell accumulations which enclose areas of the RPE-Bruch's membrane layer. In interphotoreceptor retinoid-binding protein induced EAU in mice, macrophages were also a predominant cell type but similar extending accumulations have not



been noticed (Caspi et al., 1990). In EAPU, the RPE is the target that is destroyed in a chronically progressing process whereas neuroretinitis is very scarce, and pinealitis is absent. By the elimination of fragments of Bruch's membrane and functionally active RPE cells the blood-retina barrier is interrupted. This is comprehensible in view of the fact that the rats develop EAPU after immunization with specific RPE membrane antigen (PEP-65), however, as yet we can only speculate about the precise mechanism that mediates these features.

### *Immune Mechanism*

It has been demonstrated that the initial events in uveitis may include ICAM-1 and MHC class II antigen expression close to the ocular target tissue (Fujikawa et al., 1987; present study). In addition, our results show that merely the use of pertussis toxin and CFA (without antigen) causes a slight increase in reactivity of ICAM-1 and (more clearly) of class II antigen above the normal levels. This may reflect the increase in activity of the immune system known to be evoked by these adjuvants. In agreement with previous reports about experimental uveitis (Whitcup et al., 1993; Chan et al., 1994), the first signs of EAPU were noticed closely before its onset, at day 7 (class II antigen) and at day 8 (ICAM-1). It has been shown that these molecules play a crucial role in the initiation of inflammatory processes (Tamatani and Miyasaka, 1990; Uchio et al., 1994).

Although human RPE cells may express mononuclear phagocyte CD68 antigen (Elner et al., 1992) we found no reactivity of rat RPE to monoclonal antibodies of the ED series. EAPU includes the migration of ED1+ macrophages and T cells through the Bruch's membrane-RPE layer. This process is preceded by activation of choroidal vascular endothelium and resident macrophages (present study), and possibly by melanocytes and RPE cells as in other experimental models of uveitis (Chan et al., 1994). None of these phenomena is observed in the neural retina which is secondarily involved in EAPU. Hence, expression of adhesion molecules occurs close to the target. Several data indicate that a macrophage flux occurs across the blood-retina barrier. Macrophages first accumulate in the choroid. Subsequently, they are observed in the Bruch's membrane-RPE layer which is dissociated and destroyed. In addition, histology as well as electron microscopy are highly suggestive for a permeation of this barrier. Finally, although extending cell accumulations are also seen subretinally, no marked extravasation of cells has been noticed at the retinal vessels which excludes these vessels as origin.

It has repeatedly been shown that CD4+ T cells act as effector cells in autoimmune diseases. In experimental autoimmune encephalomyelitis (EAE), these cells alone seem to be required to induce the disease

(Sedgewick, Brostoff and Mason, 1987). However, the role of CD4+ cells is complex because its subsets Th1 and Th2 act in a different way (Mossmann and Coffman, 1989). In addition, macrophages appear to play an important role. ED1+ phagocytes are the first inflammatory cells to appear in the uvea in EMIU (Caspi et al., 1990; Chan et al., 1994). In studies of S-antigen-induced EAU and rhodopsin-induced EAU (Broekhuysse et al., 1991b) macrophages were most frequently observed as early invaders at the target site, i.e. the photoreceptor layer. In addition, macrophage depletion has been shown to abrogate EAE (Huitinga et al., 1990), and ED1+ cells accumulate around the RPE (containing PEP-65) in EAPU which consequently is destroyed. EAPU can be transferred by spleen lymphocytes (Broekhuysse et al., 1992b) but in EAE as well as in EAPU CD4+ cells could act in a permissive manner for the influx of antigen-sensitized macrophages and non-specific inflammatory cells as described for autoimmune demyelination (Raine et al., 1990). Once this process has been initiated it may soon lose its function, explaining the observation that the small population of lymphoid cells is scattered through the uvea. In contrast to observations in EAU (Chan et al., 1986), we found no MHC class II antigen expression at the RPE in EAPU. The latter is comparable with the situation in experimental allergic encephalomyelitis (EAE) where it is neither found on the blood-brain barrier (Huitinga et al., 1993). Hence, it is not yet known in which way T cells enter the intraocular compartment. Specifically activated T cells may reside within the choroid and may induce a macrophage influx. Resident macrophages and dendritic cells (Choudhury et al., 1994) may play a role as indicated above.

A specific role for various subpopulations of macrophages in experimental autoimmune disease was recently demonstrated by Dijkstra and coworkers by using the ED series of monoclonal antibodies (references in Table I; Bauer et al., 1994). Our data for the first time indicate that these subpopulations perform a significant role in experimental uveitis as well. Infiltrating ED1+ macrophages (and consequently their effector molecules like TNF- $\alpha$ , IL-1, IL-16, oxygen radicals and proteases) are involved in RPE elimination and probably also in the observed photoreceptor damage. Although expression of ED1 immunoreactivity may suggest the presence of activated (ED1+) microglia cells in the retina we have not positively identified such cells. We cannot exclude the possibility that they become involved in EAPU like they do in experimental autoimmune encephalomyelitis (Bauer et al., 1994). The present results also indicate that the ED2+ cells are actively involved in the initiation and progression of EAPU. They become activated just before EAPU starts, and are increasingly seen when the disease develops. Uveal ED2+ cells represent resident dendritiform macrophages. These potentially antigen-presenting cells, and the OX-6 reactive



dendritiform cells (previously described by Choudhury et al., 1994) presumably play a role in regulating autoimmune responses to ocular autoantigens. Autoantigenic peptides generated by the RPE (from photo-receptor antigens and PEP-65) may reach the dendritiform cells, and presentation to autoreactive T cells could result in an autoimmune response. The results of a recent study indicate that in EAE, the increase of ED2+ perivascular cells is correlated with the infiltration of hematogenous macrophages (Bauer, 1994). Macrophages apparently play an important role as effector cells in inflammatory disease of the central nervous system as well as in EAPU where ED2+ cells increasingly were found perivascularly (most evident during the onset). The presence of small numbers ED2+ macrophages in the trabeculum, anterior chamber, vitreous, and in the subretinal fluid in case of retinal detachment shows that these cells could migrate during EAPU. About the role of ED3+ macrophages little is known. Our finding that their activity in EAPU is increased and parallels those of ED1+ and the T cells may partially be explained by recent findings. They show that rat strains susceptible to experimental autoimmune diseases appeared to exhibit a much higher ED3 expression than resistant strains, and this enhanced ED3-expression is T cell-mediated (Damoiseaux et al., 1992). As summarized previously (Huitinga et al., 1993), ED7 recognizes type three complement receptor (CR3) on macrophages and some other cell types. CR3 is involved in phagocyte adherence to endothelium and CR3-mediated phagocytosis, and treatment of rat with ED7 suppresses EAE (Huitinga et al., 1993). The extensive occurrence of ED7+ macrophages in EAPU indicates an important role for this type of cells in this form of uveitis.

#### The RPE in EAPU

The role of the RPE in the induction and development of EAPU is highly intriguing. As an essential, active constituent of the blood-retina barrier it produces cytokines and may express immunomodulating adhesion molecules influencing disease induction and development. This is in agreement with the finding that toxic damage to the RPE inhibits the development of S-antigen-induced EAU in a dose-dependent way (Konda et al., 1994). In EAPU, focal massive macrophage fluxes into the choroid and through the RPE-Bruch's membrane layer start soon after choroidal ICAM-1, and ED2+ antigen expression increases. Immediately afterwards, the RPE cells disintegrate and thus lose their active role. The specific RPE antigen PEP-65 is focally eliminated (Broekhuysse et al., 1992b) which abrogates further binding and attraction of antigen-specific inflammatory cells. This may explain why the accumulation of macrophages stops after a critical amount of these cells has collected at both sides of the RPE.

An alternative explanation of the latter phenom-

enon in EAPU is linked to the cytokine production faculty of the RPE. Pleiotropic cytokines like IL-1 $\beta$  and GM-CSF (Planck et al., 1993), IL-6 (Benson et al., 1992), and MCP (monocyte chemotactic protein) (Elner et al., 1991) evade from the RPE cell layer after receiving a signal (e.g. IL-1, or TNF- $\alpha$ ) from the activated immune system which attracts or stimulates immunocompetent cells. A similar signal may evoke choroidal ICAM-1 expression which precedes cell infiltration in EAPU indicating such early signal transmission. Inflammatory cells infiltrate the choroid and the subretinal space. The process stops by the immunologically triggered RPE cell disintegration in this disease. In this way the accumulation of macrophages is focally terminated but may continue around still functional, intact and active RPE areas. This might explain the chronic progressing process of local cell accumulation along the RPE-Bruch's membrane layer observed in EAPU. The two proposed mechanisms do not exclude each other but might be complementary.

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