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Cellular invasion on the surface of intraocular lenses. In vivo cytological observations following lens implantation

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Abstract. Cellular growth on intraocular lenses can be observed by using a specular microscope. We examined in vivo the cellular pattern on the surface of implanted lenses during the early postoperative period in 63 patients. In every case inflammatory cells were noted on the lens surface at some point during the observation period. These often showed a spindle-like appearance resembling fibroblasts. The density of the cell population increased during the following days but usually did not exceed 100 cells/mm^2. From the end of the first week, multinucleated giant cells may appear. In one case, a patient with a severe postoperative iritis and hypopyon demonstrated an extremely pronounced and long-lasting cellular proliferation.

Introduction

The general pathological principles of the inflammatory response were discovered during the last century. In the past, animal eyes have been a frequent object of research: intraocular foreign bodies and sterile inflammations create a cellular reaction in the anterior chamber. Von Recklinghausen [22] described the serrated form and active mobility of the "purulent bodies." He found them to contain "pigment granules." Conheim [6] studied the mechanism of inflammation by pulling a silk thread through the eye of an animal or by injecting anilin blue into the anterior chamber. He showed that the "purulent bodies" originated from the blood. Leber [15] inserted fine glass rods into the anterior chamber, in which traces of "pus" appeared, while the remainder of the eye showed no trace of pus.

Harold Ridley noted that pilots whose eyes were injured by fragments of shattered Plexiglas only revealed an insignificant tissue reaction. As a result of this observation, he developed intraocular lenses (IOLs) made of Plexiglas. Five of the first eight patients receiving these lenses developed an iritis that could easily be controlled by nonsteroidal anti-inflammatory medication [23]. Eyes enucleated because of problems subsequent to lens implantation were examined by pathologists at an early stage [24, 28, 30]. Because the lenses themselves had to be dissolved for histological preparation [27, 30], only the histological changes of the tissues were described. With the introduction of lens implant cytology by Wolter [34], cytological studies of explanted lenses gained broad interest. On four patients, Ohara [18] observed cell-like structures in vivo using a specular microscope. His findings on the surface of intraocular lenses in the living eye corresponded with Wolter's observations in explanted IOLs.

In order to describe the early cellular response we examined implanted IOLs immediately after surgery and followed up their course during the first 2 postoperative weeks.

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Patients and methods

Patients

Sixty-three patients from a random population of patients undergoing cataract extraction with lens insertion at the Department of Ophthalmology at the RWTH in Aachen were examined during the first few months of 1986. The mean age of the patients was 70 years. In all cases, the operative method was extracapsular extraction with a Sinskey-style posterior chamber lens implant. Immediately after surgery a subconjunctival injection of depot prednisolone was given. During the following days, the patients received a combination of dexamethasone and neomycin topically and 2 × 50 mg diclofenac systemically.

On average, the lenses were examined with the specular microscope three times during the first 2 postoperative weeks. In our clinic, cataract patients are generally inpatients until the 5th postoperative day. Because some patients lived at a great distance from the clinic, they were not available for a regular check-up. Therefore, we did not examine much more than one-third of them during the 2nd postoperative week (Figs. 1 and 2). In Figs. 1 and 2 the cell density is summarized: day 1 (1–2), day 3 (3–4), day 5 (5–6), day 7 (7–8), day 10 (9–11), and day 13 (12–15). We saw 10 patients between 16 and 30 days after implantation, some of them even several times up to 1 year after the operation.

Technique

The implanted lenses were observed using the Biophtal microscope manufactured by Leitz with the lowest magnification (× 120). For photographic documentation at 100-W mercury flashlight and a 200 ASA slide film (Kodak Ektachrome) were used. The contrast could be enhanced by turning down the aperture of the light source. As the angle between the plane of observation (lens surface) and objective of the microscope is 22.5°, the restricted depth of field

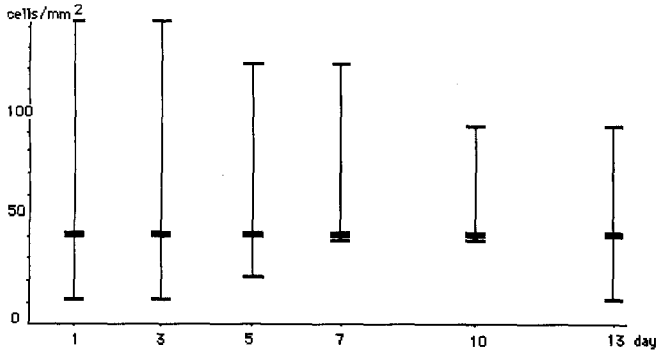

Fig. 1. Fibroblast-like cells on intraocular lenses. Time course of cell counts on the surface of implanted intraocular lenses in the case of $n=63$ patients (day 1, $n=53$; day 3, $n=57$; day 5, $n=51$; day 7, $n=19$; day 10, $n=23$; day 13, $n=12$). Median and 25%–75% quantile

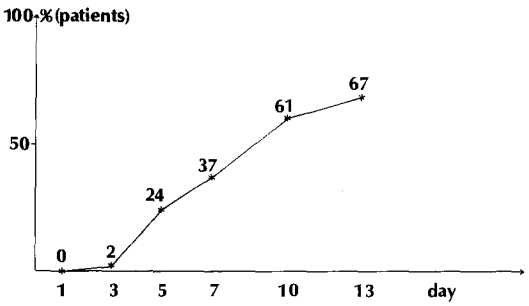

Fig. 2. Giant cells on intraocular lenses. Percentage of patients who had giant cells on the surface of implanted intraocular lenses in the case of $n=63$ patients (day 1, $n=53$; day 3, $n=57$; day 5, $n=51$; day 7, $n=19$; day 10, $n=23$; day 13, $n=12$)

rendered pictures that were somewhat out of focus peripherally. Continuous cooperation of the patient was necessary. The pupil had to be dilated to enable evaluation of the peripheral areas of the posterior chamber lenses. Applying the noncontact method, we initially focused the tear film of the cornea and then shifted posteriorly onto the lens. If the patient looked straight ahead and the lens was situated exactly parallel to the pupillary plane, the lens appeared as a bright dazzling reflex. If the lenticular plane was not parallel to the plane of the objective of the microscope, the patient was asked to move his eyes carefully in the necessary direction until the specular area had been found. After localizing that specular area, other parts of the lens could be investigated as well. Examination of the entire lens surface was facilitated by having the patient direct his gaze in different directions. This was the main problem in performing specular microscopy; since most of the eye movements were not careful enough and the specular reflex was lost from the area of observation. The cellular distribution was uneven in many cases, mainly in later stages. Therefore, patients should be preexamined with a slit-lamp.

In the examination of IOLs, two different methods of illumination were possible: direct and indirect specular microscopy. Direct specular microscopy was the examination of reflexes off the anterior lens surface. Due to the convexity of the lens surface, the examined areas appeared as round disc-like areas with brighter central illumination. Indirect specular microscopy involved examination of the anterior lens surface using light reflected from the posterior lens surface, similar to retroillumination photography. Since the posterior lens surface was plano, the whole area was illuminated evenly. In contrast to direct specular microscopy, indirect specular microscopy could be difficult – even in patients who were very cooperative – because of the difficulties in finding the specular area on a planosurface.

Evaluation

The pictures taken in this study were evaluated using the fixed-frame technique. A square frame corresponding to a lenticular area of 400 μm × 400 μm (0.16 mm^2) was projected onto areas that were easily amenable to evaluation. The cells were counted within these borders. Several areas were examined and the mean values calculated. We tried to count between three and ten fields per patient, but in some cases where there was insufficient cooperation it was impossible to count more than one. Groups with a certain range of cell density were created. The most frequent result was about 30–40 cells/mm^2 or 5–6 cells within one frame of observation. The largest differences between the single quantifications of multiple observers were 30%, mainly if there were few cells on the lens (less than 50 cells/mm^2). Only cell structures with a diameter of more than 10 μm were counted (Figs. 3–6a–c). Smaller structures such as pigment granules (Figs. 5, 6d) or erythrocytes (Fig. 6a), as well as appearances not related to cellular structures (e.g., residues of lens matter, scratches on the lens), were disregarded.

Results

To examine the cell content, indirect microscopy was preferred, but direct specular microscopy allowed better evaluation of the cellular surface. Specular microscopic examinations of the posterior lens surface commonly produced unsatisfactory results since the posterior lens surface was not as exposed to the cellular precipitations. Irregularities of the tear film caused by lipid droplets following application of ointment had a disadvantageous effect on the image quality and produced disturbing shadows on the lens. Pronounced corneal opacification or clouding in the anterior chamber were also disruptive and made the examination almost impossible. There was no correlation between the number of inflammatory cells in the anterior chamber of the eye and the cells on the surface of the lenses.

Differentiation of types of cells

In the specular area different kinds of structures could be demonstrated. During the first postoperative days, the most commonly observed cells were small translucent structures with a diameter of 10–20 μm. Frequently intracellular brown inclusions were noted. Initially, the shape of the cell was often round or oval resembling raindrops on a window. Later, the cells acquired a spindle shape with 2–3 projections. The cell length measured up to 150 μm (Figs. 3, 5, 6c). Following other authors [1, 16, 18, 40], we termed these cells *fibroblast-like cells*. A second type of cell exhibited a flat, round, map-like form. Under direct specular microscopic examination they appeared homogeneously gray. The projected light was sometimes separated by the smooth cytoplasma of the cells into its colorful prismatic

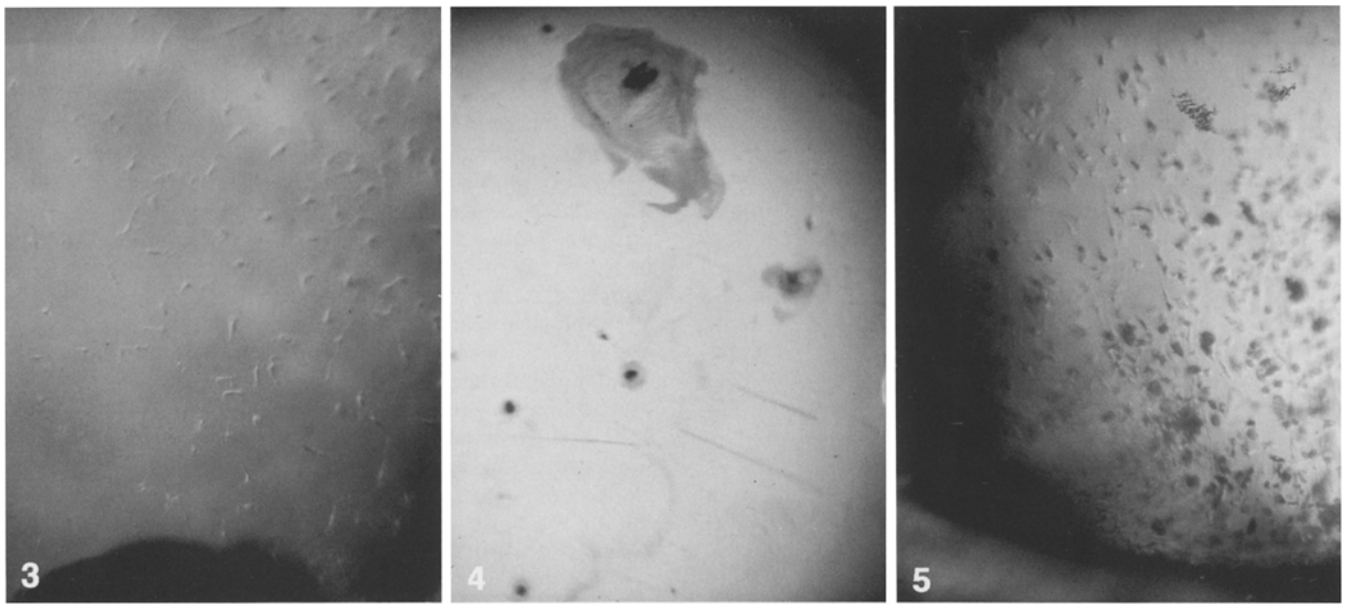

Fig. 3. Fibroblast-like cells on the surface of an intraocular lens 4 weeks after implantation. The cell count (150/mm^2) is still within the limits of a normal postoperative course (× 100)

Fig. 4. Giant cells and a few smaller epitheloid-like cells on the surface of an intraocular lens, a typical cellular appearance 8 weeks after implantation (× 100)

Fig. 5. Fibroblast-like cells in the area of pigment debris 5 days after implantation. The concentration of the cells exceeded 400/mm^2 near the pupillary area, but was less in the central area of the lens (× 100)

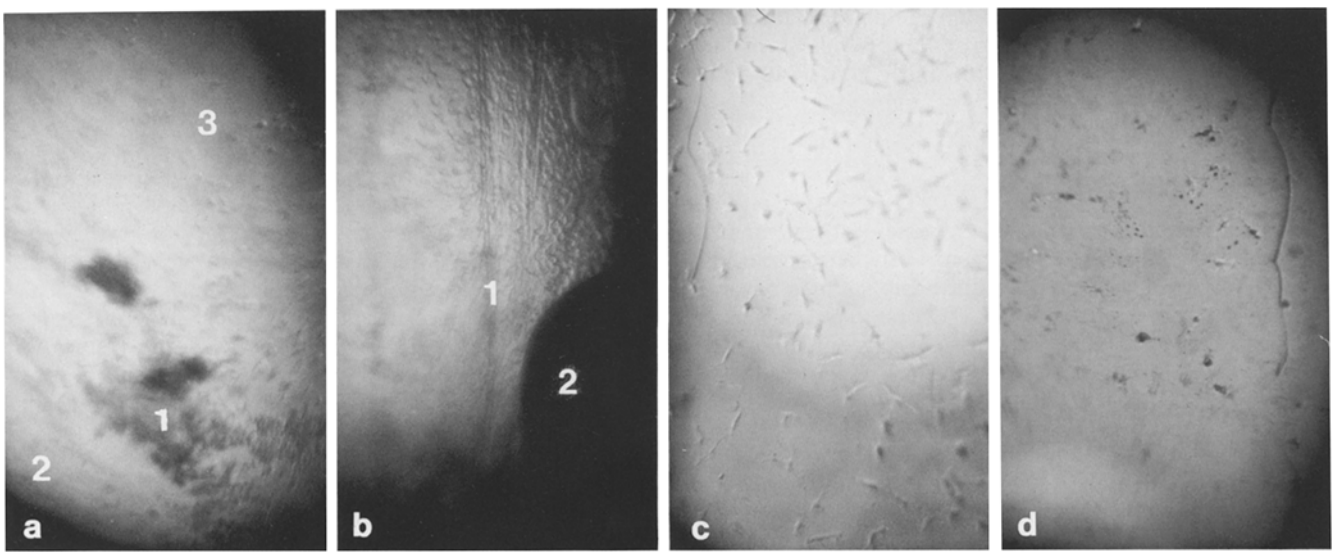

Fig. 6a–d. Cell pattern on the surface of an implanted lens in a patient with a severe postoperative iritis (× 100). **a** Brown deposits resembling erythrocytes (1), a grey smudgy film (2), and inflammatory cells with a density of 180/mm^2 (3) on the first postoperative day still appeared to be within the limits of a normal postoperative course. **b** Many small cells and strand-like formations (1) on the 13th postoperative day afer the severe iritis. Positioning hole of the lens (2). **c** Three months later. Fibroblast-like cells, 100/mm^2. **d** One year later. The fibroblast-like cells have disappeared

colors. According to the wavelength of the mercury light, the cytoplasma at these parts is not much thicker than 1 µm. Under indirect specular microscopic examination, the plane revealed a clear and transparent appearance. Centrally, the cells contained small, darkly granulated structures (nuclei). The size of the cells was variable, with a diameter ranging from under 20 µm to more than 200 µm. Smaller cells up to 40 µm we termed epitheloid-like cells, larger ones we termed *giant cells* (Fig. 4). Differentiation from the fibroblast-like cells was not easy in all cases, since a few epitheloid-like cells showed spindle-like projections as well.

Fibroblast-like cells

As early as the 1st postoperative day small, fibroblast-like cells were found on the lens surfaces of 49 of the 53 patients

examined. On day 5, no lens remained without cells. The density of the cell population varied between 1 and more than 400 cells/mm^2. The cell density showed a wide dispersion with no uniform pattern. Over two-thirds of the patients revealed a cell density of less than 100 cells/mm^2 with a mild day-to-day variation.

Generally, there was little change in cell density during the observation period. Between day 1 and 3, 49% showed no obvious change and 37% showed a moderate increase in cell density. In 14% of the patients a decrease in cell density was seen. Comparing the densities of the 5th and 7th day, only minimal elevation was found. Patients with initially high densities showed decreasing values, while patients with low initial densities tended towards increasing values.

Giant cells

Giant cells and epitheloid-like cells appeared at a later stage (Fig. 2). By the 4th postoperative day, giant cells with a size of about 80 µm were observed on the lens of only one patient. On the 7th day occasional giant cells could be demonstrated on one-third of all lenses (Fig. 2). Epitheloid-like cells, which had a diameter of less than 40 µm, developed just as slowly and appeared to be precursors of the giant cells. At the end of the study, more and more patients were found to have giant cells (Fig. 2).

We saw ten of the patients for a longer period than 2 weeks. Eight of them showed giant cells on the lenses between 16 days and 1 month after operation. The size of the cells also increased. The medium diameter of the cells was 44 µm (40–60) on the 5th day, 64 µm (40–160) on the 7th, 115 µm (40–300) on the 10th, and 228 µm (40–500) on the 13th day.

Cell pattern in a patient with postoperative iritis

Of particular interest is the clinical course of a 45-year-old patient who had developed a hypopyon-iritis postoperatively. Surgery took place with no major complications. Specular microscopic examination on the first day showed a smeared, translucent film on the lens, accompanied by large, brown deposits resembling clusters of erythrocytes. A dense growth of small, rather undifferentiated fibroblast-like cells of approximately 180 cells/mm^2 (Fig. 6a) appeared to be within the limits of a normal postoperative course. Specular microscopic examination was impossible 2 days later due to a severe hypopyon-iritis with marked opacifications in the anterior chamber. Under intense steroid therapy, the inflammation slowly improved. Specular microscopic examination could be performed on the 13th postoperative day. At this time, the lens was densely covered by small fibroblast-like cells, exceeding 400 cells/mm^2 (Fig. 6b). In the pupillary region fibrous strands were noted on the lens surface. During the following months the number of cells declined slowly. Their length increased and the typical fibroblast-like form of cells resulted (Fig. 6c). Occasionally, giant cells could be demonstrated. In contrast to the fibroblast-like cells, the development of giant cells was unremarkable. One year after surgery almost no cells were observed on the lens surface (Fig. 6d). Compared with other patients with uncomplicated courses, we found only diffuse remnants of pigment deposits on the lens surface.

Discussion

Cytological examinations of lens explants

The existence of cells on the surface of intraocular lenses has been reported only in a few studies [9, 14, 28, 29, 30]. It was not until recently that the examination of cellular deposits on explanted specimens became routine. For this purpose, Wolter described a simple staining procedure [34]. Most of the explanted lenses showed a cellular growth. Nevertheless, Wolter did not regard the occurrence of cells as a pathological reaction, but assumed that a complete covering of a lens implant by a monolayer of an optically clear cellular membrane would be highly desirable [36]. On the other hand, the same cells are essential components of dense and obstructive scaring as well as granulomatous inflammation, two of the common findings in complicated lens implant surgery [37].

On the surface of artificial lenses Wolter mainly described histiocytes in their different reactive states. There are two typical forms of differentiation: giant cells and small cells resembling active fibroblasts [36]. It is not unusual for foreign body giant cells on intraocular lens implants to grow to a diameter of about 500 µm. It is common to see all stages of their development from single sessile histiocytes to epithelioid cells and finally to giant cells [35]. An essential task of both giant cells and fibroblast-like cells is phagocytosis [40].

Apart from histiocytes, Wolter described different types of cells on IOLs. Among these are erythrocytes [40], endothelial cells [38], and polymorphonuclear leukocytes [36]. Uveal melanocytes might occur but are difficult to distinguish from fibroblast-like cells using morphological criteria [34].

Other investigators who described the cytology of explanted lenses obtained similar results. Sugar et al. [29] and Siepser and Kline [26] found structures resembling endothelial cells on the surfaces of their samples. Apple [2] reported fibroid layers and the sporadic occurrence of erythrocytes and remnants of inflammatory cells. Daicker [7] distinguished between spindle cells and giant cells.

Using scanning electron microscopic criteria, Sievers and von Domarus [27] were able to discern histiocytes, fibroblasts, foreign-body giant cells, and melanocytes. Bryan et al. most commonly found mononuclear inflammatory cells, followed by spindle cells. Other deposits on explanted lenses were found to be polymorphonuclear leukocytes, giant cells, and acellular fibrous structures [5].

Goder and Völker-Dieben [10] described melanocytes, histiocytes (i.e., macrophages, epitheloid cells, giant cells, fibroblasts) and endothelial cells. In one case it was obvious that epithelial cells of the cornea spread from the operation wound onto the surface of the lens, i.e., an epithelial downgrowth. Kappelhof et al. [13] found in three out of nine cases flat cells that supposedly represented corneal epithelial cells. Puck et al. [20] made a distinction between iris pigment epithelial cells, fibroblasts from the iris stroma, inflammatory cells, lens epithelial cells, erythrocytes, myofibroblasts, and corneal endothelial cells. They also managed to culture some of these cells. Furthermore, Ratner [21] found acellular, inorganic particles on lenses before implantation. Often these particles were not visible through the operating microscope and only a few showed a diameter of more than 40 µm. The larger particles could easily be

washed away and therefore would not remain on the lens after the operation. Thus, these particles would most likely not play a significant role in creating artifacts during in vivo specular microscopy. Bene and Kranias [3] described a possible source of contamination from talcum powder from surgical gloves, but they did not prove this theory by cytological examination. Another microscopical aspect of explanted lenses is the biodegradation of loops as described by Drews and Smith [8].

While most of the cytologically examined lenses were situated in the eye for a long period of time, Wolter and Kleberger [39] reported the result of an examination performed on the lens of a patient who died from a myocardial infarction 7 days after implantation. Until then, the eye had shown an uncomplicated healing process. On the surface of this lens macrophages and erythrocytes were isolated. Similarly, when Bryan et al. [5] studied a group of 53 explanted lenses, they included some lenses which had remained in the eye for only a short time. On the surface of one lens, mononuclear inflammatory cells were found at 1 day after the operation. On another lens they described polymorphonuclear leukocytes on the 3rd postoperative day. Schlote et al. [25] also found polymorphonuclear granulocytes 2 days after lens implantation.

Specular microscopic examinations of lens implants

Until now specular microscopes have rarely been used for in vivo examination of the cytology of implanted intraocular lenses ([18] and for further literature see Ohara [19]). The contact systems were originally constructed for the purpose of corneal endothelial diagnostics [4]. In order to observe more posterior parts of the eye, special contact elements might be necessary [17]. Using the noncontact method, the lens can be examined without significant difficulties. Vogt was the first to describe this technique of specular microscopy of the eye (for further literature see Vogt [31]. The Biophtal microscope allows both types of examination. We preferred the noncontact method because it is less disturbing to the patient. The corneal reflex does not hinder the observation as it does in noncontact endothelial microscopy.

We have two indications that the structures here interpreted as "cells" are living cells: we were able to compare specular microscopic results of a lens with the cytologically stained preparation, having biomicroscopically examined the lens shortly before explantation. In this way the specular microscopic criteria of multinucleated giant cells and fibroblast-like cells were defined [33]. We also examined the movement of the cells. All cells revealed alternative periods of migration and rest. During a half-hour period, about one-third of the cells moved while two-thirds remained immobile. However, all cells had moved in the course of the day except for giant cells with a diameter of more than 250 μm. The average speed of the cells was about 1 μm/min. There were no significant differences between the movement of fibroblast-like cells and epitheloid-like cells [32].

Without exception, all lenses observed in this study were covered with cells within a few days after implantation. The cells appeared to be similar in many cases. Different patients showed differences in the development of cell density on the lenses. Many patients never had more than 40 cells/mm^2, while others had more than 400 cells/mm^2. To date, the reason for these differences remains unknown. These postoperative observations have to be interpreted critically, as the cellular density might be different in different areas of the lens. Therefore, the given densities (Fig. 1) did not necessarily represent the total lens surface, but corresponded more with cell densities in certain areas of the lens. Technical reasons would make an examination of the surface of the total lens very cumbersome and would require an unreasonable amount of cooperation from patients coming for a routine investigation.

Many distinct interpretations of the cells were made by the authors cited. The majority of cells in this study were histiocytes. Initially, the cells were relatively small and separated from each other. They grew in number and size during the first few days. This would not be typical for either endothelial cells, iris pigment epithelial cells, or melanocytes. Many cells showed brown inclusions in their central area. These most likely represent phagocytized material, e.g., pigment or remnants of erythrocytes. Many cell types are capable of phagocytosis such as granulocytes, macrophages, and fibroblasts. Polymorphonuclear neutrophil leukocytes are cells derived from the blood with a short life span, and they have no capability of further differentiation. In cultures of human blood they die rapidly [11, 12]. On explanted lenses, these cells were detected most frequently in acute inflammatory processes [5, 34].

Histiocytes were the most common and important type of cells on the surface of artificial lenses in our study. The term "histiocyte" comprises all types of cells which develop from monocytes (mononuclear inflammatory cells) of the blood. They have a life span of several months. Monocytes leaving the blood stream initially become macrophages that are capable of developing into other cells [16]. Macrophages can be the origin of either smaller epitheloid cells or larger multinucleated giant cells [11]. Using morphological criteria, macrophages themselves might appear as fibroblast-like cells, and they remain capable of changing into other forms. They also have the capacity to develop into true fibroblasts [1].

This study did not succeed in identifying special types of inflammatory cells. For now we have decided to classify the cells into no more than two major groups. The plain, gray cells were termed epitheloid-like cells if their diameter was less than 40 μm. Larger cells were classified as giant cells. On the other hand, the small, translucent cells with an oval or spindle-shaped form were named fibroblast-like cells, in accordance with the literature cited above. These seem to be macrophages, but they also may be fibroblasts or polymorphonuclear neutrophil leukocytes. In rare cases they might represent melanocytes or other types of cells.

This study was not designed to further differentiate cell types cytologically on IOLs. However, it offers the possibility of directly observing the progress of the cytologic reaction in humans in a noninvasive fashion. In contrast to the cytological studies performed to date, information may be gained not only about cases requiring an explantation of the intraocular lens but also about the so-called uneventful postoperative courses. In the case of severe iritis, the in vivo cytology did not seem to be of great value in prediagnosing the unfavorable course, but it may be of great value to find out the definitive end of the inflammatory reaction. The macrophages stayed much longer on the implant than other leukocytes used to stay in the anterior chamber of the eye. The description of cytologic reactions obtained by using the specular microscope may supple-

mented present-day knowledge about the inflammatory process after lens implantation.

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