The effects of experimental uveitis on anterior uveal prostaglandin transport and aqueous humor composition

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The intravitreal injection of bovine serum albumin (BSA, 10 mg. per eye) or bacterial endotoxin (0.01 mg. per eye) produces classical signs of uveitis in the rabbit eye. Profound iritis and ocular hypotension could be observed within 6 to 12 hours after the injection, at the same time the aqueous humor is invaded by white cells, its [ascorbate] decreases and [protein] increases. The present study shows that parallel to these developments, there is a severe inhibition of the active accumulation of tritiated prostaglandins ([\textsuperscript{3}H]-PG's) by the isolated anterior uvea. Following endotoxin injection, all but the last of these parameters return toward normal between 7 to 14 days, while a recovery of apparent prostaglandin transport capacity requires several more days. In BSA-induced uveitis, an initial, partial recovery of all these parameters is superseded by a second wave of inflammation. Subsequently, most of these parameters show some recovery, but hypotension persist for up to 10 weeks and anterior uveal [\textsuperscript{3}H]-PG accumulative activity did not recover during the 20-week duration of this study. Such a prolonged, and possibly permanent, loss of anterior uveal PG transport capacity may be responsible for, or contribute to, the known vulnerability of the eye, which suffered one episode of severe uveitis, to recurrence of inflammation.

Key words: prostaglandins, prostaglandin transport, uveitis, iridocyclitis, inflammation, intraocular pressure, aqueous humor, ascorbate, endotoxin, albumin.

It is the mark of a great man that a true appreciation of all his contributions requires a perspective, the perspective of distance or the perspective of time. It was one of Dr. Smelser’s uniquely clear and quietly stimulating lectures that attracted me to Ophthalmology Research, but having spent most of my scientific career in his research group, I began to take his vast knowledge and the availability of his advice for granted. It is only now, that he is no longer with us, that I fully realize the great influence he had on all of us in ophthalmology and in particular on those of us who had the privilege of being associated with him. I am pleased, therefore, to be able to contribute this particular manuscript, which Dr. Smelser read only a few weeks before his death, to the George K. Smelser memorial issue.

The problem of uveitis has been studied
over several decades by many outstanding investigators, including Dr. Smelser, as new concepts of cellular and chemical mediation of inflammation evolved. The most recent development in this area is the apparent involvement of prostaglandins (PG's) in the inflammatory processes and the demonstration that nonsteroidal anti-inflammatory agents are inhibitors of PG synthesis.

It has been known for some time that irritation of the iris results in the release of potent vasoactive substances, later identified as a mixture of PG's. More recently, it has been shown that intracamerally injected or topically applied PG's can cause iritis, ocular hypertension, exudation of proteins into the anterior chamber, and an increased permeability of the iridial blood vessels to fluorescein. Furthermore, in both acute untreated anterior uveitis in human and in experimental immunogenic (BSA-induced) uveitis in the rabbit, there are very large accumulations of PG's in the aqueous humor.

PG accumulation in the intraocular fluids may be the result of increased local synthesis and release, and/or a decrease in the normal rate of loss of PG's from these fluids. It has already been argued that the rapid PG removal across the blood-intraocular fluid barriers is due to a facilitated PG transport mechanism. The primary site of this transport mechanism appears to be the ciliary processes since anterior uvea could be shown to concentratively accumulate tritiated PG's ([3H]-PG's) in vitro. Such anterior uveal [3H]-PGE, accumulation in vitro is completely blocked at the peak of BSA-induced experimental uveitis.

The present experiments were undertaken to define the onset and duration of BSA-induced inhibition of uveal PG transport and to correlate these changes with the changes in some other parameters which can be expected to be altered during inflammation. Bacterial endotoxin-induced uveitis was also studied to determine whether these effects on PG accumulation are unique to immunologic processes or represent a more general response to uveal inflammation.

Materials and methods

New Zealand white (albino) rabbits (body weight 2 to 4 kilograms) of either sex were used. Topical anesthesia (0.5 per cent tetracaine hydrochloride) was applied before all manipulations of the eye and the animals were killed with an overdose of Nembutal immediately before enucleation.

Sterile bovine serum albumin (BSA) solution (30 per cent w/v) was obtained from Gallard-Schlesinger Chemical Manufacturing Corporation (Carle Place, N. Y.) and was further diluted with sterile saline to obtain a 100 mg. per milliliter solution. The propert globe was entered with a 30-gauge needle near the equator and 0.1 ml. of BSA solution was injected into the center of the vitreous body in about 10 seconds allowing another 10 seconds before the withdrawal of the needle. The contralateral control eyes were injected with 0.1 ml. of saline in the same manner.

Shigella endotoxin (Lipopolysaccharide B, Shigella flexneri) was obtained from Difco Laboratories (Detroit, Mich.). It was made up in saline to a concentration of 0.1 mg. per milliliter; 0.1 ml. of this solution was injected into the vitreous body as described before. The contralateral control eyes were injected with 0.1 ml. of saline.

In vivo observation. Intraocular pressure (IOP) was measured with a floating tip pneumatic tonometer probe which was connected to regular laboratory recording equipment. The animals were restrained in rabbit boxes and were allowed to calm down for at least 30 minutes before measurements. The results are expressed as the IOP of the experimental eye relative to that of the control eye measured at the same time (IOPexp./IOPcont.). The hyperemia of the iris was judged on the basis of the color of the tissue and engorgement of blood vessels and was rated 0 through ++.

Studies on intraocular fluids. At the termination of each experiment, aqueous humor, and in some cases, also the vitreous humor (using 25-gauge and 18-gauge needles, respectively), were removed. Red and white cells were counted using a hemocytometer. The [protein] was determined by the Lowry technique. For ascorbic acid determination, 0.1 ml. of 5 per cent metaphosphoric acid was added to 0.1 ml. of sample to precipitate the proteins; duplicate 0.05 ml. aliquots of the supernatant were titrated with 2,6-dichlorindophenol.

In vitro prostaglandin accumulation. The globe was bisected at the level of the ora serrata; the iris, together with the ciliary body, was removed,
rinsed in saline, lightly blotted on wet filter paper, and transferred into 5 or 10 ml of tissue culture medium (Eagle's basal medium, Microbiological Associates, Inc., Bethesda, Md.) containing a trace amount of 5,6-[3H]-PGE, (New England Nuclear Corporation, Boston, Mass.), representing a total [PGE] of approximately 10^-8M. The incubation fluids were bubbled with a 5 per cent CO2-95 per cent O2 gas mixture prior to and during the one-hour incubation period. After incubation, the tissues were lightly blotted on wet filter paper, weighed on a Cahn electrobalance (Model 7500), and were transferred into scintillation vials containing 10 ml of Aquasol (New England Nuclear Corporation). The vials were shaken overnight and then counted in a Packard Tri-Carb scintillation counter to obtain a minimum of 5,000 to 10,000 counts. Aliquots (0.1 ml) of the incubation medium were prepared and counted in the same manner. Some 3H samples were burned in a Packard Model 306 Tri-Carb automatic sample oxidizer before counting.

The active fraction of 3H accumulation by the anterior uvea of the experimental eye relative to the contralateral control eye was expressed as \((T/M - 1)_{\text{exp.}}/(T/M - 1)_{\text{rest.}}\). The \(T/M - 1\) was used to indicate the active accumulation since even maximally inhibited anterior uvea shows a \(T/M\) of approximately unity.

**Results**

**BSA-induced uveitis.** Within 4 to 12 hours following the intravitreal injection of 10 mg of BSA, iritis developed, the IOP gradually decreased, the [protein] of the aqueous humor greatly increased while its [ascorbate] decreased and large numbers of white cells appeared in this normally cell-free fluid (Fig. 1, A, panels B through F). In addition to these classical signs of ocular inflammation, the present studies show that anterior uveal PG transport activity, as measured by in vitro [3H]-PGE, accumulation, was essentially abolished (Fig. 1, A, panel A) at the onset of the inflammatory response.

Some recovery of these initial effects was evident by the third day after the BSA injection. Between 6 and 10 days, a second inflammatory response became evident. The [protein] and white cell count of the aqueous humor rose again (Fig. 1, A, panels D and F), the intensity of iritis increased, while the aqueous humor [ascorbate] and the anterior uveal [3H]-PGE, \(T/M\) again became depressed (Fig. 1, A, panels B, E, and A). Typically, the IOP was slightly elevated for several days during this second inflammatory reaction (Fig. 1, A, panel C). The first reaction occurred within the first 24 hours in almost all rabbits and the time of onset of this reaction was essentially synchronous, while the onset of the second reaction typically occurred anywhere between 6 and 10 days following BSA injection (Fig. 2). The mean values in Fig. 1, A represent the general time course of uveitis, the time course of iridial hyperemia, and of IOP changes following the intravitreal injection of BSA in one typical animal is shown in Fig. 3.

The second inflammatory response lasted for several days following which there was a gradual diminution of iridial hyperemia and aqueous [protein] and the eye again became hypotonic. The [ascorbate], IOP, and the [3H]-PGE, accumulation, however, remained depressed for several weeks after visible signs of uveitis (hyperemia) disappeared (Fig. 1, A). Partial recovery of several of these parameters was evident between 4 to 10 weeks, although the [protein] remained slightly elevated throughout the 20-week period of this study, by which time the IOP reached normal value, and the [ascorbate] also showed substantial recovery. The most profound residual effect at the end of the 20-week period was the depression of anterior uveal PG accumulative capacity. This depression of anterior uveal PG accumulative capacity did not show a clear trend of recovery and, in fact, one of these rabbits which was killed eight months after BSA injection still showed an almost complete inhibition of anterior uveal PG accumulative activity.

**Endotoxin-induced uveitis.** The effects of intravitreal injection of 0.01 mg of Shigella endotoxin are essentially the same as the initial changes occurring after injection of 10 mg of BSA. The major difference between BSA and endotoxin-induced uveitis is that in the latter case, there was no second inflammatory response; instead, re-
Fig. 1, A. The effects of intravitreal injection of 10 mg. of bovine serum albumin (BSA) into rabbit eyes. Panel A: in vitro [3H]-PGE$_i$, accumulation: The isolated anterior uvea was incubated for 1 hour at 37° C. in culture medium containing [3H]-PGE$_i$. The relative extent of anterior uveal PG accumulation is expressed as (Exp T/M - 1)/(Cont T/M - 1), where T/M = [(3H per millgram of tissue)/(3H per microliter medium)]. Panel B: The extent of iritis based on the color of the iris and on the degree of engorgement of iridial blood vessels. The maximum iridal inflammation observed in this study was rated as 3+. Panel C: The IOP of experimental eyes relative to the IOP of contralateral control eyes. Panel D: The concentration of soluble proteins in the aqueous humor of experimental eyes. The mean [protein] of all contralateral control eyes is shown by the dotted line near the baseline. Panel E: The relative (Exp./Cont.) ascorbic acid concentration in the aqueous humor. Panel F: The white cell count of the aqueous humor of experimental eyes. White cells were not observed in control eyes. Points with limits (± one standard error) represent mean values obtained on four or more (in case of Panels B and C, up to 26) rabbits. Points without limits are individual values except for panel F where the mean values obtained on two to four rabbits are given without limits. B. The effects of intravitreal injection of 0.01 mg. of Shigella endotoxin into rabbit eyes. See legend to Fig. 1, A for description of each panel.
covery of all the parameters studied was observed within a few weeks. Visible signs of iritis disappeared by the twelfth day, by which time the aqueous humor [protein] also returned to normal (Fig. 1, B, panels B and D), while the [ascorbate] and the IOP returned to normal only by the fourteenth day, and white cells did not completely disappear from the aqueous humor before the eighteenth day (Fig. 1, B, panels E, C, and F). Normal anterior uveal PG accumulative activity is even slower to recover; in fact, of all parameters studied, this is by far the last one to return to normal level following endotoxin-induced uveitis (Fig. 1, B, panel A).

Discussion

The present results confirm our previous observations that during the acute phase of BSA-induced anterior uveitis, the ability of the iris-ciliary body complex to actively accumulate [3H]-PGE, in vitro is blocked. Furthermore, this study shows that such an effect is not limited to BSA-induced uveitis, but it is also a consequence of bacterial endotoxin-induced, nonimmunogenic uveitis. These findings suggest that a blockade of the anterior uveal PG transport mechanism, which under normal conditions prevents the entry of PG's from the posterior segment of the eye into the anterior chamber, may contribute to the accumulation of PG's in the aqueous humor during the course of acute anterior uveitis.

The most striking new finding of the present investigation is the very early onset and the prolonged duration of this blockade of PG accumulative capacity following BSA injection.

Although the occurrence of some inflammatory response within the first 24 to 48 hours following intravitreal antigen injection has been observed by several investigators, their interest was focused on the true immunologic response and they gave only a cursory description of this initial phase of BSA-induced uveitis. Since a true immunologic response cannot be expected to occur within the first few days after the introduction of an antigen into a nonsensitized animal, it has been assumed that this initial response to BSA is due to the trauma of the injection. In the present experiments, the control eyes of all rabbits (to date, more than a hundred animals) were injected with saline, yet iritis or any other sign of ocular inflammation was never observed in a control eye. Thus, this first response must indeed be due to the injected protein, or to a contaminant of the protein preparation, rather than to the trauma of the injection itself.

Preliminary experiments (L. Z. Bito, unpublished observations) show that this initial inflammatory reaction occurs with several different commercially available preparations of bovine, rabbit, or human serum albumin. One possible explanation is that commercial preparations of serum albumin contain significant amounts of endotoxins. This tentative conclusion is supported by the fact that under identical experimental conditions, a pyrogen-free pharmaceutical solution of human serum
Fig. 3. The time-course of BSA-induced iritis and IOP changes in one typical rabbit. See also legend to Fig. 1, A, panels B and C.

albumin does not cause a similar initial episode of uveitis.

It remains to be seen what role, if any, the initial inflammatory response may play in the development of the true allergic component of the BSA-induced uveitis. Endotoxins are known to enhance antibody formation and the initial inflammatory response, whatever its etiology may be, may greatly influence the distribution and disposition of the intravitreally injected antigen. It should also be noted that the reversibility of the sequelae of uveitis may depend on both the severity and the duration of the inflammation. Following BSA injection, overlapping of the two episodes of uveitis can result in 14 to 20 days of an essentially continuous state of inflammation, as compared to the 4 to 7 days of iritis following the fully reversible endotoxin-induced inflammation.

The question also remains whether the observed inhibition of in vitro PG accumulative capacity has a selective effect on PG transport mechanisms or is just an expression of a more general effect of inflammation on uveal metabolism, transport functions, and/or permeability. During the onset of uveitis, the aqueous humor [protein] increases and [ascorbate] decreases more or less simultaneously with the depression of uveal PG accumulative capacity. All three of these effects could simply be explained on the basis of increased leakiness of the blood-aqueous barrier. During recovery, however, these parameters are dissociated. By the tenth day after endotoxin injection, the aqueous humor [protein] is essentially back to normal and the [ascorbate] and IOP also return to normal by the fourteenth day; whereas the anterior uveal PG accumulation does not return to normal until after the third week. Recovery of normal [ascorbate] and [protein] in the aqueous humor are similarly dissociated from PG accumula-

*It should be noted, however, that an alternate, and possibly better, explanation for the decrease in aqueous humor [ascorbate] is a decrease in ciliary body blood flow. Endotoxins, and inflammatory processes in general, are known to lead to regional hemostasis which can also explain exudation of proteins, lowered secretory rate and, consequently, ocular hypotony.
relative capacity following the second episode of BSA-induced uveitis.

It has been known for some time that many eyes which suffered one episode of uveitis remain altered for a long period of time, or possibly permanently. One expression of this alteration is an increased vulnerability to the recurrence of uveitis. It has been argued, on the basis of convincing evidence, that the underlying mechanism is an essentially permanent increase in the permeability of the blood-aqueous barrier to macromolecules such as proteins. The present results do not disagree with this conclusion since even though the [protein] in the aqueous humor was observed to return toward normal level 4 to 5 weeks after an episode of severe uveitis, it remained slightly elevated throughout the duration of this study. However, the most profound, long-lasting, and possibly permanent, effect observed on these BSA-injected eyes appears to be the abolishment of normal anterior uveal PG accumulative capacity.

The suppression of normal absorptive PG transport may be a key factor in the vulnerability of such eyes to repeated breakdown of the blood-aqueous barrier and to recurrence of uveitis, since blockade of absorptive PG transport across the blood-aqueous barrier of the anterior uvea can be expected to render the eye more vulnerable to the intraocular accumulation of PGs and accumulation of these potent autacoids in the aqueous humor may be an important factor in the pathogenesis of anterior uveitis.

I wish to thank my colleagues, the late Dr. George K. Smelser, and Drs. Kenneth E. Eakins and Wladyslaw Manski, for the many stimulating discussions during the course of this project. The assistance of Erica V. Salvador, Ann S. Zaragoza, and Roger Baroody is also gratefully acknowledged.

REFERENCES


