Effect of x-irradiation on the development of immunogenic uveitis

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Rabbits receiving an intravitreal injection of egg albumin develop a spontaneous immunogenic uveitis about 7 days later. If, 24 hours prior to antigen injection, the rabbits are given 450 r of x-irradiation to the body with the eyes shielded, the ocular inflammatory response is suppressed. X-irradiation of the eyes alone with the rest of the body shielded does not inhibit the development of uveitis. These data indicate that in this primary response to antigen, active sensitization of ocular tissues does not occur to any appreciable extent, and that the inflammatory cells which infiltrate the uveal tract originate extracellularly.

It is generally conceded that the classical "horse-serum uveitis," based upon an immunologic sequence of events, has provided the best experimental analogue of recurrent nongranulomatous uveitis yet to be offered. But to admit that a disease is due to an allergy or hypersensitivity does not necessarily imply a full understanding of its pathogenesis, since there are a number of different immunologic mechanisms which may contribute to such inflammatory conditions.

This form of experimental immunogenic uveitis has been studied frequently in the past. In brief, if an antigenic substance is injected into the vitreous of a rabbit, there develops after a lag of a week or so a severe "spontaneous" nongranulomatous uveitis. After this inflammation has subsided, no residual effects can be seen clinically in the majority of eyes, but there remains nevertheless the very specific "memory" of the antigen first used; the animal is now said to have a local ocular hypersensitivity. Later exposure to the same antigen, by either intracocular or extracocular routes, will cause a rapid re-development of severe nongranulomatous uveitis. If, on the other hand, antigen is injected initially into the anterior chamber, then local ocular hypersensitivity develops in the absence of the early inflammatory reaction.

It is clear, then, that this experimental ocular disease is mediated by immunologic factors. Less clear, however, is the question of which mechanisms are involved, and to what extent, in the development of the inflammatory responses. Since the original lesion develops in the eye in response to intraocular antigen injection, and since only the eye will respond with an inflammatory reaction to subsequent intravenous challenge, it is reasonable to inquire whether or not the ocular tissues play an active role in these reactions. Are the inflammatory cells within the eye derived from mesenchymal cells of the ocular tissues in loco, or is the eye only a passive...
terrain upon which the immunologic reaction takes place? In the present paper we report the results of a study on the origin of the cells involved in the primary inflammatory response to intraocular antigen, taking advantage of the fact that x-irradiation will depress or abolish the immune response to antigen.

Materials and methods

The experimental scheme employed in this study was relatively simple. In a group of adult albino rabbits (4 to 6 pounds) of the Hartley strain 2 mg. of crystalline egg albumin dissolved in 0.1 ml. of sterile saline was injected into the vitreous of the right eye as previously described. This dose almost invariably induces the development one week later of spontaneous uveitis in the injected eye, the fellow eye remaining uninflamed.

Twenty-four hours prior to such an intravitreal injection, a second group of rabbits under Nembutal anesthesia was given a single exposure to 450 r of irradiation to the body, with the top of the head and eyes shielded and thus unexposed to the x-rays. In this way, the spleen and all lymph nodes including the preauricular were irradiated. A third group of anesthetized animals received 450 r of x-irradiation to the eyes, with the rest of the body shielded from the x-ray beam. Again, the eye-irradiated animals received the same standard injection of egg albumin 24 hours after irradiation as did the other two groups. All three groups of animals were then followed clinically with slit lamp and ophthalmoscope for the next two weeks to observe whether or not uveitis developed, and between the fourth and twenty-first day after injection animals were sacrificed and the right (prinoculated) eyes taken for histologic study, as described elsewhere. Among the eye-irradiated animals, a number of uninoculated left eyes were taken at varying times to serve as controls for evaluation of the histologic changes due only to the x-ray treatment itself.

Irradiation was performed employing a G.E. Maxitron 300 instrument with an inherent filtration of 4.7 mm. of beryllium. It was operated at 350 KVP and 20 Ma., using a 2 mm. copper filter. The half value layer of the filtered beam was 3.0 mm. copper. With the rabbit lying on its left side, the dose rate for the whole-body radiation (eyes shielded) was 54 r per minute, at a distance to the center of the body of 85 cm.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X-IRRADIATION</th>
<th>ANTIGEN INJECTION</th>
<th>OCULAR REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NO IRRADIATION</td>
<td>2mg. EGG ALBUMIN O.D.</td>
<td>%/a 0.0.% INFLAMED</td>
</tr>
<tr>
<td>2</td>
<td>X-RAY, 450 r.</td>
<td>EYES SHIELDED</td>
<td>2mg. EGG ALBUMIN O.D.</td>
</tr>
<tr>
<td>3</td>
<td>X-RAY, 450 r.</td>
<td>EYES SHIELDED</td>
<td>2mg. EGG ALBUMIN O.D.</td>
</tr>
</tbody>
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Fig. 1. The experimental scheme of the radiation studies, showing the effect of irradiation to different areas of the body on the development of uveitis following intravitreal injection of antigen.
For the irradiation of the eyes, the dose rate was 317 r per minute, at 35 cm. distance. A total dosage of 450 r of x-rays has been shown to inhibit effectively the immunologic response of the rabbit to antigenic stimulus.

Results

A general outline of the experimental procedure and the results obtained is presented in graphic form in Fig. 1.

Among the control animals that received no irradiation prior to the injection of egg albumin into the vitreous, all but one of the 19 animals so treated developed a severe iridocyclitis on the sixth or seventh day following injection. This form of uveitis has been so well described before that it warrants little comment. It is, briefly, a severe uveitis, predominantly confined to the anterior segment, but with an inflammatory component at the optic nerve head. From the outset, it is composed exclusively of round cells—lymphocytes and histiocytes. At about 4 days after onset, the cell type alters somewhat, and there appear numbers of immature plasma cells. Thenceforth, mature plasma cells constitute an important component of the inflammatory infiltrate. The inflammation observed clinically generally lasts about a week to 10 days, and usually subsides without detectable sequelae.

Of the animals that had received x-irradiation to the eyes 24 hours before the 2 mg. dose of antigen was injected into the vitreous, 23 of 24 yielded essentially the same form of spontaneous uveitis as did the controls. With respect to both the time of onset of the inflammation and its average severity, no detectable difference could be found between this group of rabbits and the control group. Again, the histologic picture of the inflammatory response in the irradiated eyes was in no obvious qualitative or quantitative way different from that seen in the controls, except that in this case the immunogenic inflammatory response was superimposed upon tissue alterations due to the effect of the x-rays, and was of no special interest to the present discussion.

Quite different were the results observed in the animals that had received, 24 hours prior to intravitreal injection of antigen, the x-irradiation to the whole body with the eyes shielded. Of 23 animals treated in this fashion, only one developed a clinically apparent uveitis, on the eighth day. The remaining 22 rabbits showed no evidence of clinical involvement using either the slit lamp or ophthalmoscope.

In support of the clinical findings, histopathologic examination of the eyes of a number of the animals which failed to react also showed an absence of any appreciable cellular infiltrates of the type usually observed in the nonirradiated controls. At first glance, therefore, the eyes appeared to be normal. In almost every instance, however, a closer examination revealed that there were in fact a few round cells diffusely infiltrating the anterior uveal tract and around the vessels at the optic disc. These were fairly sparsely distributed lymphocytes and monocytes, hardly present in sufficient numbers to justify the term inflammatory infiltrate, but definitely not present in the uninjected control eye. Neither could they be attributed to the effects of the trauma of the original injection a week or two previously, since this was found not to persist past the first few days.

Discussion

It has been demonstrated repeatedly that the injection of antigen into the eye results in the local formation of antibody within the ocular tissues. Other immunologic mechanisms, however, also contribute to the development of immunogenic uveitis. Woods has suggested that recurrent nongranulomatous uveitis in the human is due, in the main, to a state of delayed, or "microbial" allergy induced by the antigenic products of organisms which have found their way into the eye. But even simple protein antigens such as egg albumin appear to be capable of inducing this form of hypersensitivity state, and we have suggested elsewhere that at
least the early stages of the spontaneous uveitis which follows the intravitreal injection of antigen may be a delayed hypersensitivity inflammation.

Granting that many of these inflammatory reactions are immunologic in nature, it has not been demonstrated whether ocular tissues contribute the cells involved in the inflammatory infiltrates, or whether the uveal tract is merely a passive field upon which extraocular cells impose when they respond to antigenic stimulus. We have sought to investigate this question, restricting the present study only to the spontaneous or primary immunogenic uveitis which follows intravitreal antigen injection.

There exists an extensive literature on the effect of x-irradiation on the immune response\(^7\) which justifies the conclusion that an adequate dose of x-rays will suppress almost completely the development of specific response to antigens. This dose in the rabbits is about 450 r of whole-body irradiation, given 24 hours before antigen. This effect has been employed in this study to answer the question of whether the sensitization by antigen in the eye is entirely a local affair, or alternatively whether it is the result of a systemic sensitization arising in the spleen and lymph nodes. If the former possibility were the case, then x-irradiation of the eyes with the rest of the body shielded should abolish the response, while whole-body irradiation with the eyes shielded should have no effect. If, on the other hand, extraocular tissues are involved in the sensitization, then irradiation of the eyes should be without effect, while whole-body irradiation should suppress the ocular inflammatory response.

The data presented in this paper show that in fact local ocular irradiation does not interfere with the development of uveitis in response to intravitreal antigen, while whole-body irradiation with the eyes shielded does suppress the clinical response. The data are so clear-cut on this point that it must be concluded that sensitization arises in the extraocular (lymphoid) tissues, and that circulating cells invade the eye in response to the attraction of residual antigen which, leaking only slowly from the depot in the vitreous, is able to persist in the eye during the period in which sensitization develops. While it is clear that hematogenous cells are responsible for the clinical uveitis, the origin of the few inflammatory cells which do appear in the eye of the whole-body irradiated animals is not known. These may represent either the slight degree of immunologic competence left to the lymphoid tissue after irradiation, or alternatively a true differentiation of autochthonous ocular cells. Since they were observed only in the inoculated eye, it may be concluded that they were not related specifically to radiation but to an immunologic response.

Whether the recurrent uveitis which accompanies a later intravenous challenge of the prestimulated animal is also of extraocular origin will be the subject of future studies. It should, however, be pointed out that in the latter instance ocular inflammation results even though antigen is not presented directly to the previously inoculated eye. In this case, we might predict that the condition known as local ocular hypersensitivity may actually involve immunologically competent cells arising within the eye.

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REFERENCES


