The effects of cortisone on pine pollen-induced uveitis in guinea pigs

Stan L. Coleman and Samuel Canaan

Injection of pine pollen into the vitreous of guinea pigs previously sensitized with Freund's adjuvant and pine pollen intradermally and intraperitoneally resulted in a disseminated granulomatous inflammatory process involving the entire uveal tract. Moderate or large doses of steroids (2 to 8 mg. per kilogram per day of prednisolone) diminished the reaction when given 1 to 2 weeks after the challenge, but caused an increase in severity of the reaction if given during the period of sensitization or immediately after the challenge.

It has previously been shown that pine pollen, after suitable subcutaneous sensitization, is capable of inducing granulomatous reactions when administered systemically or intracamerally. The present study demonstrates the production of granulomatous lesions in the eye by intravitreal injections of pine pollen in sensitized guinea pigs. The effects of systemic steroids on the reaction are also described.

Methods and results

Pigmented and nonpigmented guinea pigs, weighing 300 to 500 grams were used in this study. The pine pollen was from loblolly pines collected in the vicinity of Macon, Georgia.

The animals were examined clinically by means of the slit lamp and the ophthalmoscope.

Experiment A. Clinical and histologic study of intravitreal pine pollen. Initially the guinea pigs were divided into three groups:

1. Nonsensitized animals. Pine pollen* injected by means of a 27 gauge needle into the vitreous of one eye of 5 nonsensitized guinea pigs evoked no clinical reaction. Histologic sections were within normal limits. These eyes were observed for periods up to six months and monthly histologic sections were obtained. The pine pollen could be seen in the vitreous, but was free of reaction.

2. Freund's adjuvant alone. Pine pollen was injected into the vitreous of one eye of 10 guinea pigs 2 weeks after sensitization with 0.5 ml. of Freund's adjuvant alone (0.4 ml. intradermally and 0.1 ml. intraperitoneally). This caused a reaction after 3 to 4 days consisting of conjunctival injection and mild vitreous haze. This disappeared after an additional 3 to 4 days,

*Because of its severely hydropic nature, the exact amount of pine pollen used per animal in this study could not be determined.
and monthly histologic sections for a 6 month period of observation revealed no abnormalities except for lymphocytic conjunctival infiltration.

3. Freund's adjuvant plus pine pollen. Pine pollen was injected into the vitreous of one eye of 10 guinea pigs after sensitization with 0.5 ml. of Freund's adjuvant plus pine pollen (0.4 ml. intradermally and 0.1 ml. intraperitoneally). This resulted after 3 to 4 days in a reaction similar to that of Group 2, but persisted and increased in severity.

Clinically there were conjunctival injection, ciliary flush, moderate anterior chamber cells and flare, dilatation of iris vessels, and marked vitreal haze.

Histologically, after one month, there was massive vitreal reaction composed of pockets of round cells with a few polymorphonuclear cells, exudative retinal detachment, round cell infiltration of the ciliary

Fig. 1. Round cell infiltration of ciliary body one month after challenge. (Hematoxylin and eosin. x100.)

Fig. 2. Preretinal clumping of round cells, giant cells, and epithelioid cells one month after challenge. (Hematoxylin and eosin. x100.)
Effects of cortisone on pine pollen-induced uveitis

Volume 1
Number 6

753

body (Fig. 1), and preretinal clumping of round cells, giant cells, and epithelioid cells (Fig. 2).

After 2 months the vitreal reaction was greatly reduced, leaving a posterior lenticular membrane. There were proteinaceous material and round cells in the anterior chamber. The retina remained detached, mainly at the periphery (Fig. 3), cyclitis was still present, and the choroid revealed small areas of round cell infiltrations.

After 3 months the proteinaceous material was still present in the anterior chamber as well as the keratitic precipitates. The retina and ciliary body were as previously noted. The massive vitreal reaction had essentially cleared. The iris revealed granulomatous nodules at the pupillary margin (Fig. 4). Most of the eyes at this time were becoming phthisical.

No significant changes were noted in the opposite eye of these animals which served as controls.

Experiment B. Effects of steroids (2 mg. per kilogram per day of prednisolone). In the second stage of this study, the guinea pigs were divided into 7 groups, 9 animals per group. The method of pine pollen injections and size of the guinea pigs were the same as previously noted. Pine pollen was injected into the vitreous of one eye of 63 guinea pigs 2 weeks after sensitization with 0.5 ml. of Freund's adjuvant plus pine pollen (0.4 ml. intradermally and 0.1 ml. intraperitoneally) just as in Group 3 of Experiment A.

1. Control group, no steroids given. The clinical and histologic reactions in this group were similar to those previously described in Experiment A, Group 3, consisting essentially of granulomatous involvement of the entire uveal tract.

Groups 2 to 7. The remaining groups of animals received prednisolone, 2 mg. per kilogram per day intramuscularly, begun as follows and continued to the end of the experiment:

2. Treatment begun 3 days prior to sensitization.
3. Treatment begun with sensitization.
4. Treatment begun with challenge.
5. Treatment begun 3 days after challenge.
6. Treatment begun 1 week after challenge.
7. Treatment begun 2 weeks after challenge.

In Groups 2 to 5, the reaction began similarly to that of the control group, but became much more severe in a matter of 7 to 8 days. The eyes went on to phthisis after one month.

Histologic sections at this time revealed complete retinal detachment and severe anterior chamber reaction with proteinaceous material and round cells, giant cells, and epithelioid cells. After 2 months the vitreal reaction was greatly reduced, leaving a posterior lenticular membrane. There were proteinaceous material and round cells in the anterior chamber. The retina remained detached, mainly at the periphery (Fig. 3), cyclitis was still present, and the choroid revealed small areas of round cell infiltrations.

After 3 months the proteinaceous material was still present in the anterior chamber as well as the keratitic precipitates. The retina and ciliary body were as previously noted. The massive vitreal reaction had essentially cleared. The iris revealed granulomatous nodules at the pupillary margin (Fig. 4). Most of the eyes at this time were becoming phthisical.

No significant changes were noted in the opposite eye of these animals which served as controls.

Experiment B. Effects of steroids (2 mg. per kilogram per day of prednisolone). In the second stage of this study, the guinea pigs were divided into 7 groups, 9 animals per group. The method of pine pollen injections and size of the guinea pigs were the same as previously noted. Pine pollen was injected into the vitreous of one eye of 63 guinea pigs 2 weeks after sensitization with 0.5 ml. of Freund's adjuvant plus pine pollen (0.4 ml. intradermally and 0.1 ml. intraperitoneally) just as in Group 3 of Experiment A.

1. Control group, no steroids given. The clinical and histologic reactions in this group were similar to those previously described in Experiment A, Group 3, consisting essentially of granulomatous involvement of the entire uveal tract.

Groups 2 to 7. The remaining groups of animals received prednisolone, 2 mg. per kilogram per day intramuscularly, begun as follows and continued to the end of the experiment:

2. Treatment begun 3 days prior to sensitization.
3. Treatment begun with sensitization.
4. Treatment begun with challenge.
5. Treatment begun 3 days after challenge.
6. Treatment begun 1 week after challenge.
7. Treatment begun 2 weeks after challenge.

In Groups 2 to 5, the reaction began similarly to that of the control group, but became much more severe in a matter of 7 to 8 days. The eyes went on to phthisis after one month.

Histologic sections at this time revealed complete retinal detachment and severe anterior chamber reaction with proteinaceous material and round cells, giant cells, and epithelioid cells.
Fig. 5. Severe choroiditis showing nest of round and epithelioid cells in cortisone-treated animal one month after challenge. (Hematoxylin and eosin. x450; reduced \( \times \).)

cceous material plus numerous cells. There was a severe cyclitis and a choroiditis consisting of discrete areas of epithelioid cells and round cells (Fig. 5). There were no iris nodules.

In Groups 6 and 7, the reaction was less severe than in the control group. Histologic sections after one month revealed a clear anterior chamber, mild cyclitis, and less choroidal involvement. The retinas revealed exudative detachment in the periphery.

**Experiment C. Effects of large doses of steroids (8 mg. per kilogram per day of prednisolone).** In the third stage of this study 6 guinea pigs were sensitized and challenged as previously noted in Experiments A, 3, and B. Daily treatment with prednisolone intramuscularly in doses of 8 mg. per kilogram per day was begun concurrently with sensitization and continued to the end of the experiment.

Clinically there were conjunctival injection, clear anterior chamber, and a vitreous haze which prevented visualization of the fundi. Histologic sections after one month were similar to those of the animals treated with lesser doses of prednisolone (Experiment B, 2 to 5) except for the absence of anterior chamber changes.

**Discussion**

A picture of disseminated granulomatous uveitis is produced by pine pollen in guinea pigs previously sensitized systemically with Freund's adjuvant and pine pollen. By injection of the pollen into the vitreous rather than intracameraly, the reaction involves the entire uveal tract rather than being confined to the iris.

Since systemic and ocular sarcoid are treated clinically with steroids, it was of interest to evaluate the effects of steroids on this experimental granulomatous uveitis. In this study animals benefited from steroids when therapy was begun one and two weeks after the reaction was initiated. This would simulate the clinical situation of partial suppression of inflammatory response by such therapy.

The groups of animals (B, 2 to 5, and C) treated earlier in the course of the disease or before its production, and with moderate or large doses of steroids, all developed a much more severe disease process. One can only speculate as to the mechanism of this greater reaction. It resembles superficially the dissemination and progression of some infectious granulomatous processes (e.g., tuberculosis) and may relate to reduced "resistance" to the disease process.

It has been shown that steroids not only enhance antibody production in the guinea pig but produce an effect that resembles the consequences of administration of thorotrast, trypan blue, India ink, or iron saccharate on the reticuloendothelial system.

The colloidal substances appear to interfere with the ability of the reticuloendothelial system to remove, fix, or detoxify certain products. The result may be, according to Thomas, "a shifting of these functions to tissues not normally concerned with them, with uncontrollable tissue damage."7

We wish to thank Mr. Edward G. Locke, Chief, Division of Wood Chemistry, United States Department of Agriculture, for supplying the pine pollen necessary for this study, and Mrs. Mildred Williams and Mrs. Yvonne Gathright for their technical assistance.
REFERENCES


