UVEAL TISSUE AS AN ADJUVANT TO LENS PROTEIN

H. S. DUA AND ISHWARCHANDRA

Department of Ophthalmology, Medical College and Hospital, Nagpur

ABSTRACT

The lens proteins are weakly antigenic and require the help of an adjuvant to produce antibodies. Bacterial proteins and toxins have been vairously cited as adjuvant *in vivo* although there is no convincing proof for this. The present work has demonstrated experimentally that whereas antibodies are not produced on injection of only lens or only uveal extract, they are produced when a mixture of lens and uveal extracts is injected into rabbits. The present work has also revealed that the uveal protein or pigment acts as an endogenous adjuvant in the producing antibody response.

INTRODUCTION

THE antigenic property of the lens was discovered in 1903[1] and it was later established that this antigenicity was responsible for the condition termed 'Endophthalmitis phacoanaphylactica'[2]. It is well established that the lens protiens are weakly antigenic and fail to produce any significant antibody response unless administered along with an adjuvant[3–8]. Burky[3] demonstrated the adjuvant action of staphylococcal toxins, and since then several workers have recorded that bacterial antigens and toxins act as 'adjuvant *in vivo*'[4–11].

In experimental animals also the necessity of using Freund's complete adjuvant[12–13] has been amply confirmed[11,14–17]. Muller's[7] comment on this issue sums up the consensus of opinion: "Low grade infection in humans which clinically might go unnoticed might possibly play the role of an adjuvant. Living or dead micro-organisms or toxins might act as adjuvant and in combination with liberated lens material lead to sensitisation and eventually to a phacogenic ophthalmia. Adjuvant may enter at the time of operation or by blood." It is evident that this explanation has been offered and accepted merely for want of a better one, since none of the workers was able to demonstrate a convincing source of bacterial toxins, whereas all workers merely presumed the presence of a hidden septic focus[3,7,11].

The present investigation was undertaken with a view to adducing experimental evidence to arrive at a more plausible explanation to this phenomenon. Further, during clinical work the present authors noticed that during extracapsular extractions there was often a considerable dispersal of uveal pigment while washing the anterior chamber of the eye and more so when pieces of capsule were pulled out with an Arruga's forceps. This led to the suspicion that the pigment or proteins from the uvea might be acting as an endogenous adjuvant to the lens protein to augment its effect.

EXPERIMENTAL

The experimental procedure was as follows:

1. Human cataractous lenses and goat uvea were used for the experiments. For each experiment three lenses were homogenised in 1·0 ml of normal saline and two uvea were homogenised in 1·0 ml of normal saline. The stock antigen solutions were prepared under completely sterile conditions and this was further confirmed by incubating the solutions at 37° C for 24 hours and inoculating the samples into blood agar plates. (Sodium azide 1/1000 was used as preservative.)

2. Four rabbits were immunised with neat lens antigen, four were immunised with neat uveal antigen and five rabbits were immunised with a mixture of equal volumes of lens and uveal antigen solution.

3. The schedule of immunisation was as follows: 0·5 ml of the antigen was injected subcutaneously at two different sites, once a week for one month. The sites of injection were changed each week. During the following month 1·0 ml of the antigen solution was injected intramuscularly into the thigh with an interval of two weeks between the two injections.

Trial bleeding was carried out one week after the last injection and the serum was obtained from each rabbit. This was stored at −20° C with sodium azide as preservative.

Ouchterlony's agar gel diffusion technique was employed to detect the presence of antibodies in the sera. A 1% agar solution (Difco Laboratories, U.S.A.) was prepared in barbitone buffer (pH 8·6) and allowed to set in thin layers on glass slides. Wells (3 mm diameter) were made in the gel in pairs 5 mm apart (Fig. 1). The paired wells were charged with the solutions as follows: In each pair one well (the left one in the figure) was charged with the serum while the other (the right one) was charged with either neat lens homogenate or neat uveal homogenate. The serum of all the experimental rabbits was thus tested against

the lens and the uveal homogenate. The slides were kept at room temperature for 24 hours, humidity being maintained by keeping a wet filter paper in the petri-dish. The slides were then eluted in normal saline and kept in a refregirator for 24 hours. The uveal homogenate had consistently poor diffusion qualities in all the experiments.

Results and Conclusions

Figure 1 indicates the results of the experiments related to the reaction of antibodies with the lens homogenate. Examination of the figure leads to the following conclusions:

1. The serum from rabbits receiving neat lens antigen did not produce lines of precipitation thereby indicating that no antibodies were produced (Fig. 1-*a*).
2. Similarly the serum from the rabbits receiving neat uveal antigen also did not produce any line of precipitation indicating thereby that no antibody was produced in these too (Fig. 1-*b*).
3. The serum from all rabbits receiving the mixture of uveal and lens antigens produced one (Fig. 1-*d*) or two (Fig. 1-*c*) lines of precipitation when made to react with lens antigen. This indicates the presence of antibodies.

Reaction of antibodies with uveal homogenate was as follows:—

4. Sera of rabbits receiving neat lens and neat uveal antigen did not react with uveal antigen.
5. The serum of rabbits receiving the mixture of lens and uveal homogenate also did not show marked reaction with uveal antigen. Only a faint line of precipitation appeared in three of them.

From the above observations it is evident that individually the lens and the uveal protein are too weak to produce marked antibody response but when administered together they either potentiate each other's action or more likely the uvea augments the antigenicity of the lens protein. Probably the uvea acts as an adjuvant to lens protein as indicated by the fact that the antibodies formed in the serum after administration of the mixtue reacted better with lens protein than with uveal protein. The poor diffusion quality of the uveal homogenate does not, however, materially affect the conclusions drawn here.

Several workers[18–23] have noted a close association between sympathetic ophthalmitis (believed to be due to sensitisation to uveal pigment which is itself weakly antigenic[24–27]), and phacoanaphylactic uveitis. Blodi[22] found a 23% incidence (39/170) of phacoanaphylactic uveitis in eyes with clinically diagnosed sympathetic ophthalmitis. de Veer[18–19] suggested that phacoanaphylactic uveitis is occasionally the precursor of sympathetic ophthalmitis. He postulated that sensitisation to uveal tissue occurs following a destructive lesion produced by phacoanaphylactic uveitis. Eason and Zimmerman[23] stated—"...the almost constant occurrence of phacoanaphylactic uveitis in cases of sympathetic ophthalmitis was unanticipated and this observation requires explanation".

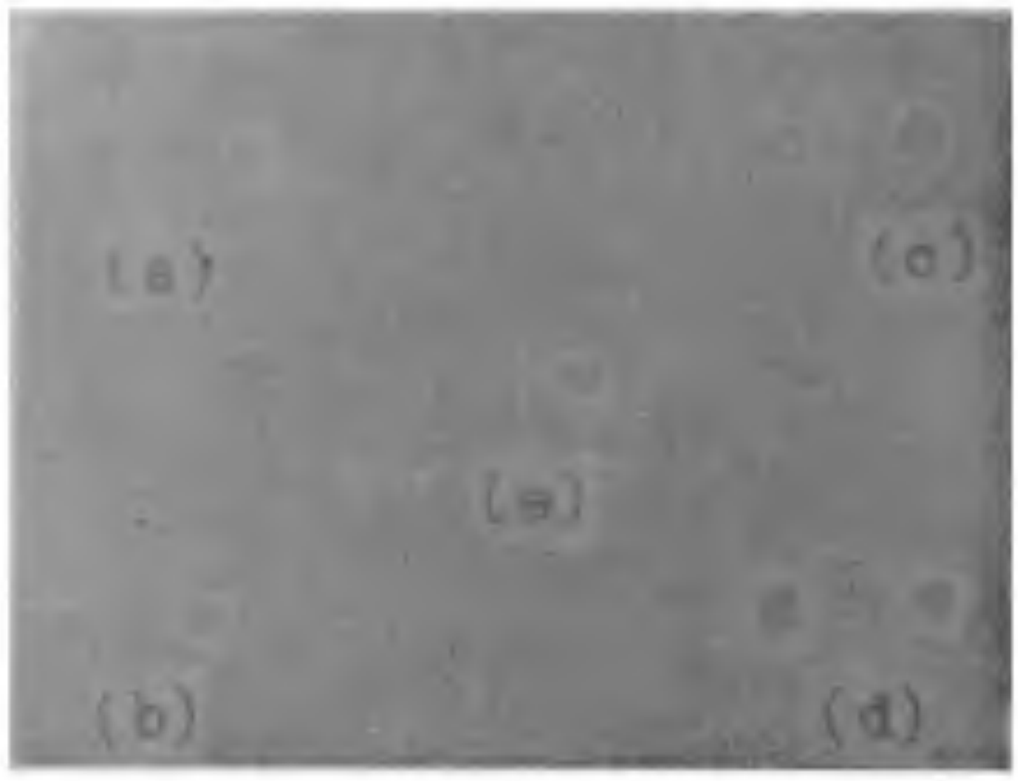

Fig. 1. Photograph of the agar gel plate 48 hours after charging the paired wells. The right well in each pair contained the lens homogenate. (*a*) The left well contained the antibodies from rabbits immunised with neat lens homogenate. (*b*) The left well contained the antibodies from rabbits immunised with neat uveal homogenate. No line of precipitation was noticed in the two cases (*a*) and (*b*). (*c*) and (*d*) The left well contained antibodies from rabbits immunised by a mixture of lens and uveal homogenate. Note the presence of one or two lines of precipitation due to antigen-antibody reaction. (*e*) Control in which the left well contained the antibodies from a rabbit immunised by a mixture of lens homogenate and Freund's complete adjuvant. Note the formation of the line of precipitation.

This explanation could well lie in the probability that the two antigens acted together, implying that the two different clinical diseases have a common basic etiopathogenesis, differing only in the nature of the response. One clinical picture dominates if the response is predominantly to one antigen whereas the other clinical picture dominates when the response is predominantly to the other antigen, although in both cases both the processes are simultaneously present. It is not unlikely that the 'perforating injury' of sympathetic ophthalmitis, which involves the iris and ciliary body, also damages the lens, at least to the extent that it may cause sufficient escape of lens proteins.

Acknowledgement

We express our sincere thanks to Dr. B. S. Richur, M.D., Professor and Head of the Department of Pathology, Government Medical College, Nagpur, for his kind help.

1. Uhlenhuth, P., 1903 (quoted by Francois, J., Rabaey, M., Wieme, R. J. and Kaminski, M., *Amer. J. Ophthal*, 1956, **42**, 577).
2. Verhoeff, F. H. and Lemoine, A. N., 1922 (quoted by Burky E. L., *Arch. Ophthal.*, 1934, **12**, 536).
3. Burky, E. L., *Arch Ophthal.*, 1934, **12**, 536.
4. Haik, G. M., Waugh, R. L., Jr., and Lyda, W., *Ibid.*, 1952. **47**, 437.
5. Scobes, R. G. and Slaughter, H. C., *Amer. J. Ophthal.*, 1956, **42**, 577.
6. Francois, J., Rabaey, M., Wime, R. J. and Kaminski, M., *Ibid.*, 1956, **42**, 577.
7. Muller H., *Trans. Ophthal. Soc. U.K.*, 1963, **83**, 687.
8. Lyda. W. and Lippincott, S. W., *Amer. J. Ophthal*, 1955, **40**, 120.
9. Brown, A., *Arch. Ophthal.*, 1934, **12**, 730.
10. Halbert, S P., Locatcher-Khorazo, D., Swick, L., Witmer, R., Segal, B. and Fitzgerald, P., *J. Expt. Med*, 1957, **105**, 540.
11. Agrawal, L. P., Brar, B. S. and Goswamy, S., *Orient Arch. Ophthal.*, 1969, 7, 294.
12. Freund, J., *Ann. Rev. Microbiol.*, 1947, **1**, 291.
13. —, *Am. J. Clin. Path.*, 1951, **21**, 645.
14. White, R. G., Coons, A. H. and Connolly, J. M., *J. Expt. Med.*, 1955, **102**, 83.
15. Halbert, S. P. and Fitzgerald, P., *Amer. J. Ophthal.*, 1958, **46**, 187.
16. Luntz, M. H., *Exp. Eye Res.*, 1964, **3**, 166.
17. Selzer, G., Luntz. M. H. and Sacks, M. I, *Amer. J. Ophthal.*, 1966, **61**, 1396.
18. de Veer, J. A., *Arch. Ophthal.*, 1953, **49**, 607.
19. —, *Ibid.*, 1940, **23**, 237.
20. Flocks, M., Litwinn, C. S. and Zimmerman, L. E., *Ibid.*, 1955, **54**, 37.
21. Kronenberg, A., *Amer. J. Ophthal.*, 1955, **40**, 205.
22. Blodi, F. C., *Trans. Amer. Acad. Ophthal.*, 1959, **63**, 642.
23. Easom, H. A. and Zimmerman, L. E., *Arch. Ophthal.*, 1964, **72**, 9.
24. Luntz, M. H., *Amer. J. Ophthal.*, 1965, **59**, 373.
25. Aronson, S. B., Hogan, M. J. and Zweiger, P., *Arch. Ophthal.*, 1963, **69**, 105.
26. Collins, R. C., *Amer. J. Ophthal.*, 1949, **32**, 1687.
27. Suie, T. and Dodd, M. C., *Ibid.*, 1955, **39**, 377.