Experimental marginal corneal infiltrates

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Marginal infiltrates arise from a wide variety of causes and according to the recent literature occur most commonly in association with chronic staphylococcal blepharitis. Marginal infiltrates often have a crescent form or coalesce to form a complete ring concentric to the limbus. On the other hand, the well-formed manifestation of an immunological process in the cornea, the so-called immunological corneal ring, often strikingly resembles marginal infiltration in man (Germuth 1959). This has given rise to the presumption that marginal infiltrate, when associated with staphylococcal lid inflammation, is a result of hypersensitivity reaction in which staphylo-antigen plays an important role (Thygeson, 1969).

Hogan, Diaz-Bonnet, Okumoto, and Kimura (1962) tested this hypothesis experimentally. Their animals were immunized with subcutaneous staphylococcal antigen with the addition of Freund's adjuvant. Immunological rings developed clinically with marked uveitis. Microscopical examination showed these infiltrates to be composed of neutrophils. The results of these experiments are very important in the studies of staphylococcal corneal disease, but the clinical appearance differs from that of a marginal infiltrate in man, because in human disease the antigen enters the organism not subcutaneously but through the cornea and/or conjunctiva, so that there is no marked uveal reaction. Also Freund's adjuvant affects the potency of the staphylococcal antigen.

The experiment presented in this paper was undertaken in an effort to produce an immunological ring using staphylo-antigen without the help of Freund's adjuvant and introducing it directly into the cornea.

Material and methods

EXPERIMENTAL ANIMALS

Fifteen chinchilla rabbits weighing from 2 to 2.5 kg. were divided into three groups of five animals. The eyes of all the animals were examined for anterior lesions before the experiment was begun.

ANTIGEN

Staphylococcal antigen was prepared from a strain of coagulase-positive *Staphylococcus aureus* isolated from the lid margin of a patient with staphylococcal blepharitis associated with marginal infiltrate. 1 g washed bacteria was suspended in 10 ml. 0.9 per cent. NaCl solution to which an equal volume of 0.15 M phosphate buffer pH 7.7 was then added. The mixture was boiled under reflux for 3 hrs and allowed to stand at 37°C. overnight. The bacterial cells were deposited by centrifugation and discarded and 2 volumes of cold ethanol were then added to the supernatant. The resulting precipitate was then redissolved in 10 ml. 0.9 NaCl solution (Hall, Smith, Edwards, and Shooter, 1969).

IMMUNIZATION

(1) Five animals were immunized by subcutaneous administration of antigen together with Freund's adjuvant for 24 days. At 3-day intervals the animals were given increasing doses of

antigen and adjuvant (beginning with 0.2 and going up to 2 ml.); 30 days after the beginning of immunization antibodies were searched for.

(2) Five animals were immunized by intraperitoneal injections. The doses of antigen and time intervals were the same as in the first group with the following differences: Freund's adjuvant was not used and booster injections were given 40, 50, and 60 days after the beginning of immunization.

(3) Five animals received intralamellar injections of 0.5 ml. antigen into the centre of the cornea.

The animals of the first and the second group were challenged by intralamellar injection of antigen after 30 and 60 days respectively. Those in the third group were not challenged.

The presence of antibodies was demonstrated by agar diffusion and precipitation technique.

Results

SUBCUTANEOUS IMMUNIZATION WITH FREUND'S ADJUVANT

After they were challenged all the animals in this group developed a more or less marked uveal reaction with fibrin deposits and cells in the anterior chamber. In two animals a semilunar infiltrate was seen separated from the limbus by a clear zone.

PROLONGED INTRAPERITONEAL IMMUNIZATION WITHOUT FREUND'S ADJUVANT

More or less severe uveitis was seen in all animals after they were challenged, but in all of them a dense, diffuse corneal infiltrate appeared at the site of injection. Three developed a ring-like marginal infiltrate after 24 hours.

CORNEAL IMMUNIZATION

All the eyes in this group were quiet until the 14th day when in two animals ring-like marginal infiltrates appeared accompanied by a few cells in the anterior chamber. The pupil was somewhat dilated and its reaction was sluggish (Fig. 1).

FIG. 1 *Appearance of eye after corneal immunization*

FIG. 2 *Plasma cells and plasmacytoid cells. $\times 360$*

The eyes were enucleated and subjected to microscopical examination. The material was stained with haematoxylin and eosin and plasma cells differentiated by the methyl-green-pyronin technique. The limbal zone of the cornea showed a heavy plasma cell infiltration. There were also many plasmacytoids in the corneal infiltrate. These cells were packed with coarse eosinophilic granules and had pyknotic nuclei (Fig. 2). The

corneal infiltrate was well demarcated at the centre of the cornea and disappeared gradually towards the limbus; there was no necrosis.

Discussion

It is apparent that the intensity and the form of immunopathological reaction in the eyes of the animals in this experiment were directly dependent on the route of antigen administration and the duration of the immunization. Marginal infiltrates appeared in those immunized with and without adjuvant, but after a much longer time in the second group. *Staphylococcus aureus* has relatively weak antigenic properties, so that either the addition of adjuvant or prolonged immunization is needed. In the human staphylococcal infection, marginal infiltrates appear after the disease had been present in the eyelid for several years.

The reactions in the systematically immunized animals differ from those in intra-corneally immunized animals. The route of administration is important in determining the form and intensity of the hypersensitivity reactions. Klinge and Vaubel (1931) discovered that the tissues which first come into contact with the antigen are first sensitized. Immunization *via* the cornea is what happens in patients with staphylococcal blepharitis whose corneae are constantly exposed to the *Staphylococcus* and its antigens. In these experiments we were also able to elicit clear-cut corneal reactions with minimal uveal irritation and this is also a feature of marginal infiltrates in man. If the antigen enters the organism through the cornea, only marginal infiltrates appear, but if the immunization is parenteral, diffuse uveal and corneal reactions are seen.

Therefore, it may be postulated that under natural conditions, in the eye suffering for a long time from staphylococcal blepharoconjunctivitis, small quantities of staphylococcal antigen constantly enter the circulation through the cornea and conjunctiva and lead to the formation of antibodies. When these antibodies reach the cornea an immunological reaction takes place and is seen as a marginal infiltrate in the otherwise transparent cornea. The presumption that corneo-conjunctival application of an antigen can lead to the state of local hypersensitivity was proved by both Chait (1950) and Aronson, Goodner, Yamamoto, and Foreman (1965).

Summary

An attempt has been made to investigate the hypothesis that marginal infiltrates may result from corneal hypersensitivity to staphylococcal antigen. It was possible to produce marginal infiltrates in experimental animals by using staphylococcal antigen. It was also possible to elicit a corneal reaction resembling that seen in man, but only if the immunization was obtained *via* the cornea; in systemically immunized animals marked uveal and diffuse corneal reactions occurred as well as marginal infiltrates.

References

- ARONSON, S. B., GOODNER, E. K., YAMAMOTO, E., and FOREMAN, M. (1965) *Arch. Ophthalm. (Chicago)*, **73**, 402
- CHAIT, R. A. (1950) *Ibid.*, **43**, 526
- GERMUTH, F. G. (1959) In "Mechanisms of Sensitivity", ed. J. H. Shaffer, G. A. LoGrippe, and M. W. Chase (Henry Ford Hospital International Symposium). Little, Brown, and Co., Boston
- HALL, J. G., SMITH, M. E., EDWARDS, P. A., and SHOOTER, K. V. (1969) *Immunology*, **16**, 773
- HOGAN, M. J., DIAZ-BONNET, V., OKUMOTO, M., and KIMURA, S. J. (1962) *Invest. Ophthalm.*, **1**, 267
- KLINGE, F., and VAUBEL, E. (1931) *Virchows Arch. path. Anat.*, **281**, 701
- THYGESON, P. (1969) *Amer. J. Ophthalm.*, **68**, 446