Phlyctenulosis

Attempts to produce an experimental model with BCG

P. Thygeson, V. Diaz-Bonnet,* and M. Okumoto

Phlyctenular keratoconjunctivitis is a clinical entity but not an etiological entity. There is general agreement that it is a manifestation of bacterial allergy, most often to products of the tubercle bacillus. The reason for the focal nature of the disease, the mechanism by which attacks are precipitated, and the all but specific role of Mycobacterium tuberculosis remain to be elucidated. Although bacterial hypersensitivity is a feature of tuberculosis and other types of granulomatous disease in various species of animals, typical phlyctenulosis never has been encountered in any laboratory animal. There are numerous claims in the literature to the production of phlyctenules in laboratory animals sensitized to tuberculin and other antigens, but it is in fact doubtful whether any experimental model of the characteristic human phlyctenular keratoconjunctivitis under discussion ever has been produced. Attempts to produce phlyctenules and phlyctenular keratoconjunctivitis in rabbits in which BCG was used as the antigen have so far failed.

Phlyctenulosis, or phlyctenular keratoconjunctivitis, is a nodular affection with characteristic clinical appearance that occurs principally in children living in poor conditions. It was originally called eczematous conjunctivitis by Blazy in 1874 because of the frequent association with eczema of the face and lids, and later scrofulous conjunctivitis by Lawrence in 1876 because the great majority of affected children suffered from tuberculosis in one or another of its many forms.

The etiology of the disease was long in dispute, but as early as 1908 Verhoeff suggested that it was a tuberculous anaphylactic phenomenon, and this hypothesis was later developed by von Szily. Other observers suggested that agents other than the tubercle bacillus could produce the same type of lesion, and experimental studies appeared to indicate that almost any protein could produce phlyctenules in laboratory animals. In the disease in human beings, however, the protein producing the sensitization has apparently been tuberculoprotein in the great majority of cases. Other sensitizing agents which have been incriminated include staphylococci, fungi (e.g., Candida albicans), and viruses (e.g., the virus of lymphogranuloma venereum). In addition to the hypersensitivity reaction, other factors are encountered in phlyctenulosis and contribute to the disease, probably as trigger mechanisms. These include vitamin deficiency, blepharitis, and acute bacterial conjunctivitis.

From the Francis I. Proctor Foundation for Research in Ophthalmology and the Department of Ophthalmology, University of California School of Medicine, San Francisco, Calif.

This work was supported by a grant from the Committee on Research, University of California, San Francisco Medical Center.

*United States Public Health Service Fellow.

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The clinical disease

The diagnostic lesion of the disease, the phlyctenule, is an inflammatory nodule composed of leukocytes and can best be described as a minute subepithelial abscess. It is in no sense a tubercle, and tubercle bacilli never have been demonstrable in histopathologic sections of the lesion or in cultures. Although the phlyctenule occasionally resolves spontaneously, it usually necroses with a sloughing of the epithelium over its apex. Early in the disease neutrophils and lymphocytes are the characteristic cells; later many plasma cells and a few mast cells appear.

For a reason not yet understood, the phlyctenules develop first and most prominently at the limbus although later they may spread over the bulbar conjunctiva and cornea. Very occasionally they have been seen on the palpebral conjunctiva. It is only on the cornea that scars are formed, and these scars with their accompanying vessels (phlyctenular pannus) are an important cause of reduced vision and blindness in many parts of the world, particularly in Alaska.

The disease occurs typically in acute, self-limited attacks lasting a week or more, but occasionally it becomes chronic. Attacks seem to be "triggered" by factors leading to an exacerbation of the underlying systemic disease, such as vitamin deficiency and malnutrition, or by factors which produce engorgement of conjunctival and limbal vessels, such as blepharitis and acute conjunctivitis. It has also been noted[6, 7] that exposure to open tuberculosis may precipitate an acute attack, presumably by reason of exposure of the conjunctiva to exogenous bacilli.

Etiology

Although the disease is not a specific etiological entity, since it seems quite certain that sensitivity to substances other than tuberculoprotein can be causal, the epidemiologic evidence clearly indicates that tuberculosis accounts for the great majority of cases. There is an almost exact parallel between the rate of tuberculosis in a community and the frequency of phlyctenulosis. In the United States, for example, it is in our Eskimo and Indian populations, in whom tuberculosis is still the major infectious disease, that phlyctenulosis is seen most frequently and in its most characteristic form.

The exact mechanism of the production of the phlyctenule is still uncertain, but it seems clear that in a child with phlyctenulosis due to tuberculosis the ocular structures have been sensitized to tuberculoprotein at an early age, presumably as part of a bacteremia from an early infection in the lungs or lymph glands. The attack is then precipitated by the presentation of tuberculoprotein to the sensitized eye tissue, either by the bloodstream in the event of a recrudescence of a pulmonary or other focus, or by an exogenous inoculation of tubercle bacilli into the conjunctival sac in the event of exposure to open tuberculosis in the family.

The peculiar focal character of the disease in the absence of tubercle formation is not understood, nor why the conjunctival and corneal disease is not ordinarily accompanied by inflammation of the uveal tract, nor why spontaneous desensitization seems to occur in adult life. It is also not known why phlyctenules do not appear in other types of allergic conjunctival disease, such as hayfever conjunctivitis, vernal conjunctivitis, and contact blepharoconjunctivitis, nor why, of the various bacterial antigens that can sensitize the ocular structures, it should be tuberculoprotein that is usually responsible for the phlyctenular disease.

History of attempts to produce phlyctenulosis in experimental animals

Phlyctenulosis appears to be strictly a disease of human beings in spite of the fact that tuberculosis and other granulomatous diseases that produce significant allergies of infection are common in the animal world. Monkeys are particularly susceptible to tuberculosis, yet in more
than 30 years of work with various species of monkeys and apes, the senior author never has seen one of these animals with an active phlyctenule or the residual corneal scarring of old phlyctenules. The possibility of producing this disease experimentally should be examined since this could be a means of answering the various questions concerning its mechanism.

The literature on experimental phlyctenulosis was reviewed by Duke-Elder and brought up to date in 1958 by Theodore and Schlossman. It has been stated that the instillation of tuberculin into the conjunctival sac of tuberculous animals has produced typical phlyctenules. The same result has been claimed in animals first sensitized to horse serum and other typical proteins, and to various bacterial products, particularly products of staphylococci. No precise criteria for phlyctenule production have been set up, however, and many of the reported experimental results are unconvincing. In no instance has the production of conjunctival or corneal phlyctenules, with corneal scars and phlyctenular pannus, been documented histologically. It seems unlikely, therefore, that a true experimental model of phlyctenulosis has, in fact, been produced in animals.

In an effort to overcome this deficiency, we have undertaken a study in three parts directed toward the production of an experimental model of the disease. If successful, we should then be able to obtain information as to the immunologic mechanisms concerned. Mycobacterial products are to be used in the first part of this study; other bacterial products, including staphylococcic products, in the second part; and various purified antigens in the third part. The following report describes a preliminary study in which the rabbit was used as the test animal and BCG as the sensitizing agent.

**Experimental studies**

_Sensitization of rabbits with BCG.*_ It is well known that BCG induces a hypersensitivity to tuberculin in man and susceptible animals, and it is of special interest that in immunization programs phlyctenulosis in children has not infrequently resulted from the accidental use of BCG in tuberculin-positive children. BCG seemed, therefore, to be a good mycobacterial antigen to use in the preliminary phase of this study. No difficulty was encountered in inducing tuberculin skin sensitivity (tested by the intradermal injection of PPD*) by the injection of 0.1 c.c. of a suspension of BCG into the groins of rabbits. These animals also showed conjunctival sensitivity when tuberculin was instilled into the conjunctival sac, but the reaction was relatively mild and of short duration (24 to 36 hours).

*Attempts to produce phlyctenulosis in sensitized rabbits by means of tuberculin._ Three rabbits which showed definite skin sensitivity after the injection of BCG into the groin were challenged repeatedly by the instillation of various concentrations of tuberculin (PPD) into the conjunctival sac. Single instillations were used in some tests, multiple instillations in others. A mild, diffuse conjunctivitis was regularly produced by this method, but no focal lesions resembling phlyctenules developed. When tuberculin was injected beneath the bulbar conjunctiva near the limbus, a focal lesion was produced, but it resembled episcleritis in human beings, did not ulcerate, and did not lead to corneal disease.

*Attempts to produce phlyctenulosis by sensitizing the eye directly._ BCG injected beneath the conjunctiva near the limbus produces a benign, self-limited granuloma the size and duration of which depend in large part upon the amount of the inoculum. BCG granulomas were produced in this way in the right eyes of 6 rabbits. When the induced lesions had subsided, which required from 6 weeks to 4 months, both eyes were challenged with instillations of tuberculin (first strength PPD). Mild, diffuse conjunctivitis developed in both eyes, but again no phlyctenule-like lesion was produced in any animal.

Three of the animals were then challenged in the right eye by intracorneal injection of 0.05 c.c. of first strength PPD in the pupillary area. Again there was diffuse reaction but no phlyctenule development, and the inflammation was of short duration (36 to 48 hours). The remaining three animals were then challenged by the intracorneal injection of 0.03 ml. of a suspension of inactivated BCG. Central opaque lesions developed in each cornea and led to severe circumcorneal flush and rapidly developing pannus. Again no focal lesions resembling phlyctenules developed.

*Attempts to produce phlyctenulosis in sensitized rabbits by injection of living BCG into the cornea._ Three rabbits previously sensitized by in-
jection of BCG into the groin were challenged in the left eye by injection of 0.03 ml. of a heavy saline suspension of living BCG into the central cornea. In each instance a keratouveitis was produced, with circumcorneal flush and severe engorgement of the bulbar vessels. No phlyctenules developed at the limbus in any animal. These animals are still under observation.

Attempts to produce phlyctenulosis in sensitized rabbits by injection of killed BCG subconjunctivally. Three rabbits previously sensitized by injection of living BCG into the groin were challenged by injections of small amounts (0.1 ml. of a heavy suspension) of killed BCG beneath the bulbar conjunctiva. Focal reactions resembling phlyctenules occurred in each instance but resolved without ulceration. After resolution of the lesions, the animals were challenged by instillation of tuberculin, but only a mild, diffuse conjunctivitis resulted.

Attempts to produce phlyctenulosis by intravitreal injection of BCG. Silverstein, Welter, and Zimmerman have shown that injection of an antigen into the vitreous forms a depot of antigen which leaks out of the site slowly, allowing antigen to be present during the development of the hypersensitive state. They noted that when crystalline egg albumin was used, uveitis developed after a lag period of about a week.

In the hope that this route of inoculation with BCG might lead to phlyctenule production, the vitreous of the right eye of two rabbits was inoculated with 0.15 ml. of a suspension of living BCG by injection through the pars plana. Although both animals developed clear-cut uveitis, no focal lesions resembling phlyctenules developed. This experiment is being continued and extended through the use of progressively increasing dosages of BCG.

Discussion

It is interesting to speculate on the nature of the association of phlyctenulosis with childhood tuberculosis. Certainly the ocular disease is associated typically with exalted tuberculin hypersensitivity, and it is not uncommon to encounter vesiculation, and even sloughing, when first strength PPD is used for intradermal testing. I have been unable to gather reliable figures from the literature on possible differences between children and adults in the degree of their reactivity to tuberculin, but quantitative tuberculin testing is planned for further clinical studies on phlyctenulosis among our Indians.

As to trigger mechanisms concerned with the initiation of attacks, it seems most likely that a rise in the activity of the focus is responsible. In support of this hypothesis is our finding in Alaskan studies that acute attacks were usually accompanied by an increase in the sedimentation rate. On the other hand, clinical observations clearly indicate that in a group of children with a high incidence of tuberculin sensitivity, such as is encountered in Indian schools in our southwest, an attack of acute bacterial (Koch-Weeks) conjunctivitis has precipitated an attack of phlyctenulosis in a significant number of cases. Weeks considered phlyctenulosis specifically related to his bacillus, but since this stimulating effect is seen only in epidemics among children who are for the most part tuberculin-positive, this concept is probably untenable. It seems much more likely that the trigger effect is nonspecific, i.e., due to dilation of the limbal vessels with an increased release of antigen to the shock tissues.

It is also interesting to speculate on the reasons for the high susceptibility of the limbus to development of the phlyctenules. The limbus is also highly susceptible to another hypersensitivity disease, vernal catarrh. There is little similarity between the lesions of these two diseases, however, probably because limbal vernal catarrh represents an immediate type of reaction, whereas limbal phlyctenulosis represents a delayed type. So far, no immunologic explanation has been advanced to account for localization in the limbus or for the fact that in some cases corneal phlyctenules become the most prominent feature of the disease.

It seems evident that a very high degree of hypersensitivity to tuberculin is a feature of phlyctenulosis in human beings, and logical to suppose that the experimental production of the disease would also require a high degree of sensitivity to the mycobacterial antigen. Our failure to produce phlyctenules in the BCG-sensitized rabbit may possibly be explained on the basis of the relatively low order of
sensitivity obtained as judged by the mildness of the conjunctival reactions.

It is also of interest that human tubercle bacilli seem to be involved primarily, at least in the United States, in the populations most affected by phlyctenulosis, i.e., our Indians and Eskimos. For example, of 5,114 cultures of tubercle bacilli studied by the Alaska Department of Health between 1944 and 1955, all were of the human variety. With this in mind we plan to employ human tubercle bacilli in continuation studies. We also plan to try a variety of tuberculins for challenge. It is interesting that Kirschenbaum, Pearson, and Lorincz, in a study of tuberculids, found that old tuberculin was more valuable than PPD in demonstrating sensitivity.

Until a laboratory model of the disease in an experimental animal can be produced, only the slowest progress can be expected in unraveling the many intriguing features of phlyctenulosis.

REFERENCES

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