Brit. J. Ophthal. (1967) **51**, 843

CORNEAL HYPERSENSITIVITY TO HERPES SIMPLEX*†‡

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DELAYED hypersensitivity has been shown to be important in causing much of the damage secondary to infection, but its exact role is often unclear.

A study of the nature of the hypersensitivity seen after systemic herpes simplex infection was carried out in this laboratory by Lausch, Swyers, and Kaufman (1966), who found a cutaneous reaction in guinea-pigs to non-viable soluble herpes antigen after herpes infection. This reaction is delayed in its appearance, is characterized by a mononuclear cell infiltration at the test site, can be transferred by cells but not by serum, and is seen before circulating antibody is detectable. The question arose whether this type of delayed hypersensitivity could play a role in the ocular disease seen after herpes simplex infection?

Disciform keratitis in man can be suppressed by small doses of steroids (Kaufman, 1965). Previous studies in this laboratory (Williams, Nesburn, and Kaufman, 1965) have shown that animals systemically sensitized to herpes virus and then given dendritic keratitis frequently develop a disciform-like lesion of the cornea, whereas unsensitized animals which are similarly infected do not. These findings suggest a possible role of hypersensitivity in the development of this syndrome. It was decided to examine the ocular hypersensitivity response using conditions in the eye identical to those previously used in documenting delayed cutaneous hypersensitivity.

Methods

Herpes antigen was prepared by infecting chick embryo fibroblast (CEF) tissue cultures with virus strain HE 17 (2nd passage on CEF). Identically treated uninfected cells were kept as a control. After the development of cytopathic effect, cell sheets were scraped into the medium and centrifuged at 2,000 r.p.m. The cells were re-suspended in saline and disrupted by ultrasound after which they were centrifuged for one hour at 30,000 r.p.m. in the ultracentrifuge to remove cellular debris and whole virus particles. The supernatant of the centrifugation was dialysed overnight against saline and stored at $-60°$ C. until use, but immediately before inoculation both the sterile antigen and the control were heated at 56° C. for a period sufficient to inactivate any residual infectivity.

* Received for publication September 9, 1966.
† Address for reprints: H. E. Kaufman, as above.
‡ This work was supported in part by the National Institutes of Neurological Diseases and Blindness of the National Institutes of Health Grants Nos. NIH 2TL NB-5398 and NB-03538.

Albino Hartley strain guinea-pigs weighing 250–300 g. were sensitized by inoculation into the skin of the stomach with viable HE 17 strain virus grown in mouse brain (3rd passage) to avoid cross-tissue sensitization.

The challenge injection of soluble antigen consisted of 30 µl. injected intracorneally in one eye, while the other eye received a comparable amount of tissue control antigen.

The 22 animals were challenged at 3, 7, and 21 days after sensitization. Both eyes were removed under anaesthesia and examined histologically 8, 24, and 72 hours, and 6 days after the challenge injection. The eyes were fixed in 10 per cent. formalin solution and 5 µ thick paraffin sections were cut and stained with haematoxylin and eosin, Giemsa, and Unna-Pappenheim methyl green pyronin stains.

For cell enumeration at least fourteen sections of each eye were studied and a total of 176 sections was counted, the cell counts being made at the limbal area and peripheral cornea. Cells were studied and differentiated according to methods previously described (Martins and Raffel, 1964).

Biomicroscopical observation of the cornea was carried out at intervals coinciding with the histological examination.

Results

8 hours after injection both the challenged and control corneae were clear except for minimal haze near the needle track. Histologically some polymorphonuclear (PMN) leucocyte infiltration was present with a small number of monocytes; almost no lymphocytes were visible (Figs 1, 2, 3 and 4, opposite). The slight cellular response at this time was thought to be primarily a reaction to trauma and was similar in both test and control groups.

24 hours after injection, although the control corneae remained clear, the corneae of animals immunized between 7 and 21 days before challenge (7–21 group) were centrally oedematous to a varying degree, some having only slight oedema and others dense clouding. Histologically, in these antigen challenged groups, the numbers of PMNs had decreased and the mononuclear cell infiltration had passed its peak. In this immunized 7 to 21 group, the lymphocyte population was greatest (Figs 2 and 3).

48 hours after injection, severe to moderate central oedema and corneal clouding was present in the 7 to 21 group.

Animals challenged 3 days after sensitization (3-day group) behaved quite differently from the 7 to 21 group. In the study of systemic hypersensitivity approximately 6 days had been required after sensitization until delayed hypersensitivity became demonstrable (circulating antibody was not detectable for 10 days after sensitization). In the eye also, the demonstration of delayed hypersensitivity required an interval after sensitization (Figs 3 and 4). In the 3-day group, although a slight lymphocytic infiltration was apparent 24 hours after challenge, it was not until 48 hours after the antigen injection that central corneal oedema began to appear. Histological observation showed the lymphocytic infiltration to be still increasing at 48 hours, and the peak was not reached until 72 hours after challenge, the time of maximal corneal clouding.

By 6 days after injection, the corneae of all groups were virtually normal, and the histological infiltration had decreased by 80 to 90 per cent.

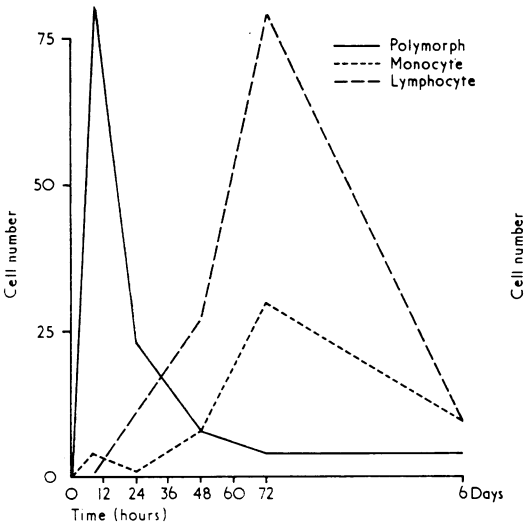

FIG. 1.—Composite graph of cells infiltrating into cornea in group of guinea-pigs challenged 3 days after being sensitized to herpes simplex virus antigen.

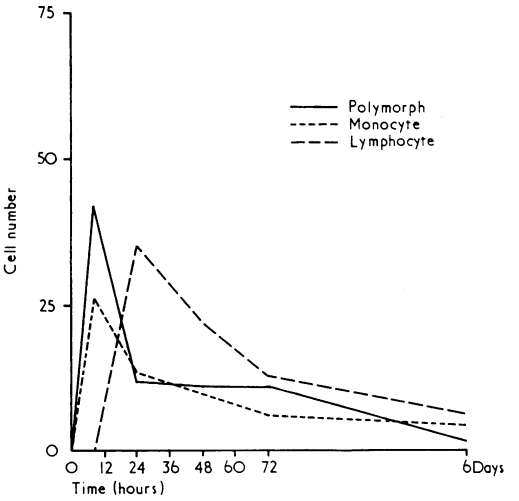

FIG. 2.—Composite graph of cells infiltrating into cornea in group of guinea-pigs challenged 7 days after being sensitized to herpes simplex virus antigen.

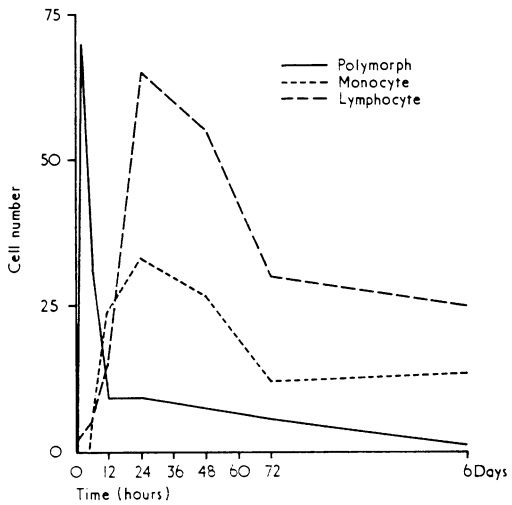

FIG. 3.—Composite graph of cells infiltrating into cornea in group of guinea-pigs challenged 21 days after being sensitized to herpes simplex virus antigen.

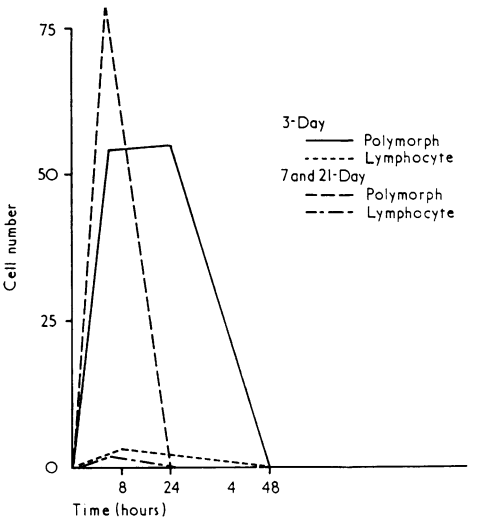

FIG. 4.—Control composite graph of cells infiltrating into cornea in groups of guinea-pigs challenged 3, 7, and 21 days after being sensitized to herpes simplex virus antigen.

Discussion

The development of corneal oedema correlated well with the infiltration of lymphocytes into the limbus. A comparison of the development of delayed hypersensitivity in the cornea with that in the skin, under identical circumstances, reveals that corneal hypersensitivity and cutaneous hypersensitivity to herpes antigen closely parallel each other. In animals immunized between 7 and 21 days before challenge, the cellular response typical of delayed hypersensitivity was demonstrable 24 hours

after challenge, but in those sensitized 3 days before challenge, severe corneal oedema and a maximal cellular response were not seen until 72 hours had elapsed. Approximately 6 days were required after sensitization for delayed cutaneous hypersensitivity to develop, and the maximal ocular response in the animals challenged 3 days after sensitization occurred after 72 hours (6 days after the initial sensitization). This close similarity between the ocular and cutaneous responses further confirms the delayed nature of the ocular hypersensitivity response.

A study such as this cannot prove that disciform keratitis is caused by delayed hypersensitivity. It does, however, demonstrate delayed ocular hypersensitivity to herpes antigen, and that this response can cause corneal oedema. It is consistent with previous studies indicating that, when animals develop dendritic keratitis, those previously sensitized to herpes antigen are likely to show corneal oedema and a swelling similar to disciform keratitis. When these results are combined with the findings of Roizman and Roane (1963) which indicated that infection with herpes simplex virus might antigenically alter the surface of the cell so that the rapid elimination of the reacting antigen, as seen in these studies, does not occur with natural infection, it seems likely that delayed hypersensitivity plays an important part in the disciform keratitis seen in man. The clinical finding that disciform keratitis can be reversed with small doses of corticosteroids also appears consistent with a delayed hypersensitivity mechanism.

Summary

A delayed hypersensitivity response was induced in the corneae of sensitized guinea-pigs in response to injections of non-viable soluble herpes antigen. This ocular response was similar in character and time of occurrence to the delayed cutaneous hypersensitivity response, and oedema and clouding of the cornea were associated with a lymphocytic infiltration at the limbus. Delayed hypersensitivity to herpes simplex virus can produce corneal oedema and may be aetiologically important in the production of disciform keratitis in man.

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