UNCLASSIFIED

AD NUMBER

AD837013

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; APR 1963. Other requests shall be referred to the Army Biological Laboratory, Attn: Technical Release Branch [TID], Fort Detrick, MD 21701.

AUTHORITY

SMUFD, per d/a ltr dtd 4 Feb 1972

THIS PAGE IS UNCLASSIFIED

TRANSLATION NO. 784

DATE: April 1943

DDC AVAILABILITY NOTICE

AD 837013

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/ TID, Frederick, Maryland 21701

FUNORESCENT ANTIBODIES IN THE DIAGNOSIS OF HERPATIC ILLNESSES

by

R. Witmer

From the University Eye Clinic in Bern (Director: Prof. Dr. H. Goldmann)

Opthalmologica 141: 278-282 (1961)

rene har a Victure e su til 24-

anion a bialbaile iligide de la

The basis of this study is the problem of the keratitides where it is not possible to determine with certainty whether there is actually still a herpatic infection or only a trophic disturbance; there are also certainly cases of herpes virus uveititis where the diagnosis can only very rarely be ascertained by the discovery of viruses in the water of the eye, because the virus cannot circulate freely in the front space because they are bound to cells.

The herpes simplex virus, although it is one of the large viruses, cannot be seen with the microscope; in humans it does not built typical enclosing bodies, but it does in the cornea of rabbits after experimental infection. Therefore the diagnosis has to be obtained from the clinical picture, which does hardly ever deceive us in fresh cases of keratitis; but in cases of deep keratitis or uncititis we often have to rely completely on suspicions.

The method of the fluorescent marked antibodies according to A. H. Coons, with which the proof of intraoccular antibody production was already made (Witmer), was already used by Vazzo and Pauluzzi in the experimental herpes infection of rabbits. They use however, a somewhat complicated socalled "sandwich technique" after Weller and Coons, with fresh serum of herpes convalescents and fluorescin marked antihuman gammaglobin.

We have chosen the direct method, as described a short time ago by H. Kaufmann for the diagnosic of herpatic keratitides of humans. Sea borses were actively immunized during several months with the viruscontaining fluid obtained from fresh tissue cultures. The antisera of

- 1 -

these animals with throughout high titers were then conjugated with fluorescin isothiocyanate. This dye has the property of binding itself strongly to protein molecules, and therefore also to antibodies, without changing their immunological properties. Under activation with ultraviolet light the antibodies which are dyes in this way fluoresce strongly and can easily be located in a fluorescent microscope. We have tried to eliminate non specific fluorescence in our experiments by adsorbing the marked antiserum with a fresh tissue culture of epithelial cancer cells.

In cell cultures of epithelial human cancer cells which are inocculated with herpes virus there is after 24 to 48 hours an already macroscopically visible cytopathogenic effect: the otherwise very symmetrical sheets of cells become irregular and often have gaps. Microscopically the normal cells, which are not infected by virus, usually have an elongated shape with a clear nucleus and often long extensions. They cannot be colored, but they have a slight proper fluorescence. The cells which are infected with virus however swell up, become grainy and absorb plenty of fluorescin antibodies. Often there is an extremely strong fluorescing zone in the near surrounding of the nucleus. The cells appear very bright, and in advanced stages often no more clear structure can be seen.

In corneal epithelia of fresh or also older herpetic keratitides the following picture is observed: besides weakly visible cells with slight proper fluorescence there are other cells which fluoresce strongly. These last cells must have absorbed marked antibodies, and therefore they must contain virus. Here also the impression is often gained that there is around the nucleus a zone of stronger fluorescence.

It is therefore possible with this method to prove the existence of herpes virus in the tissue culture and also in the corneal epithelia of clinical infections. Here we must certainly remark that in all cases which were investigated so far there was no doubt about the clinical diagnosis. So far no positive results were obtained with cells from the water of the eye, but only one questionable case of virus uveititis was specially investigated so far.

The method of the fluorescin marked antibodies is however not only suitable for finding viruses, but also just as well for bacterial or mycotic infections. Because of its great specificity this method is far preferable to the normal dying methods. It is not impossible that most germs, after they have been phagocyted by the macrophages of the water of the eye or the tissue cells of the conjunctiva have changed so much morphologically that they fail to give a typical microscopic picture. But such ruined germs still contain antigens, which can react with a specific marked antibody.

- 2 ..





a sur a sur a construction of the set of the

We believe therefore that this method will bring new interest, not only in the cytology of the conjunctiva and the cornea, (for example in the diagnosis of trachoma or epidemic kerato-conjunctivitus), but also in the cytology of the water of the eye, and that this method can open further advances in the diagnosis of the various illnesses.

In this place I must express my sincere gratitude to Dr. Wulff from the Swiss serum and vaccine institute in Bern ion her valuable cooperation.

Literature

- Coons, A. H., and Kaplan, M. H.: Localisation of antigen in tissue cells: II. Improvement in a method for the detection of antigen by means of fluorescent antibody. J. exp. Med. 91: 1-13 (1951).
- Vozza, R., and Pauluzzi, S.: Studio della charatocongiuntivite erpetica sperimentale del coniglio con la tecnica delle gammaglobuline fluorescenti. Riv. Inst. sieroter. Ital. 34:362-367 (1959).
- Weller, Th., and Coons, A. H.: Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro. Proc. Soc. biol. Med. 86: 789-794 (1954).
- Kaufmann, H. E.: The diagnosis of corneal hepes simplex infection by fluorescent antibody staining. Arch. Ophthal, Chicago 64: 382-384 (1960).

Discussion

R. Rintelen (Basel): Mr. Witmer must be congratulated for his very promising start on the way to virological investigation of the water of the eye. Our organization was clearly justified in electing him as e delegate to the international organization against trachoma.

F. Verrey (Zurich): I want to congratulate my colleague Mr. Witmer with his result. We are at the moment in the phase where data have to be collected which can only later be used for diagnosis. From my own experience I know that it is not always easy to come out of that stage. I more that in a few years this method will be widely used.

- 4 -