Fig. 1. Flux of glycine into the vitreous is shown for a control (open circles) and a light-stimulated (closed circles) animal. The bar indicates the time of stimulation with stroboscopic light at a frequency of 6 Hz.

evidence for the role of glycine as a retinal neurotransmitter.

From the Department of Neurology, Stanford University Medical Center, Stanford, Calif. This work was supported by grants NS 12079 and NS 07012 from the NINCDS, National Institutes of Health, Bethesda, Md. Submitted for publication Dec. 28, 1977. Reprint requests: Robert W. P. Cutler, M.D., Department of Neurology, Stanford University Medical Center, Stanford Calif. 94305.

Key words: glycine, retina, amino acids, vitreous, retinal neural transmitters

REFERENCES

Inhibition of macrophage migration by choroidal malignant melanoma-associated antigens in patients with uveal melanoma.

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The specific cell-mediated immunity of the lymphocytes of eight patients with choroidal malignant melanoma (MM) to four extracts of choroidal MM-associated antigens was tested with the aid of the MIF technique. Seven of the patients with choroidal MM responded to at least one of the four extracts used, whereas patients with choroidal nevus or carcinoma as well as healthy controls did not respond to any of the MM choroidal extracts. There was no response to iris extracts obtained from the enucleated eyes with MM in any of the subjects tested.

Despite the continuing refinement of modern ophthalmological tools, sometimes there are still pitfalls in the diagnosis of choroidal malignant melanoma (MM). The value of any additional specific diagnostic procedure is therefore obvious.

In the present study, the inhibition of macrophage migration (MIF) toward different extracts of primary choroidal MM was investigated in patients with choroidal MM and compared to that found in patients with choroidal nevus, patients with carcinoma, and healthy controls.

Materials and methods. The subject material comprised the following:
1. Nine patients with uveal MM. In eight patients the MM was histologically proven; six of these had choroidal MM (Patients A to F in Table I), one patient had choroidal and ciliary body MM (Patient G), and one patient had iris and ciliary body MM (Patient H). Patient I has a small...
Table I. MI's in the group of patients harboring MM versus histological diagnosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological diagnosis</th>
<th>Melanoma extract from patient</th>
<th>Iris extract from patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>A</td>
<td>Spindle A and B</td>
<td>0.65</td>
<td>0.80</td>
</tr>
<tr>
<td>B</td>
<td>Spindle B</td>
<td>0.56</td>
<td>0.52</td>
</tr>
<tr>
<td>C</td>
<td>Spindle A and B</td>
<td>0.73</td>
<td>0.67</td>
</tr>
<tr>
<td>D</td>
<td>Spindle B and epitheloid</td>
<td>1.08</td>
<td>1.14</td>
</tr>
<tr>
<td>E</td>
<td>Epitheloid</td>
<td>0.91</td>
<td>1.01</td>
</tr>
<tr>
<td>F</td>
<td>Spindle B</td>
<td>0.74</td>
<td>0.78</td>
</tr>
<tr>
<td>G</td>
<td>Spindle A and B</td>
<td>0.78</td>
<td>0.74</td>
</tr>
<tr>
<td>H</td>
<td>Epitheloid</td>
<td>0.78</td>
<td>0.74</td>
</tr>
<tr>
<td>I</td>
<td>Epitheloid</td>
<td>1.0</td>
<td>0.69</td>
</tr>
</tbody>
</table>

The MIF test was performed toward extracts of choroidal MM from the enucleated eyes of Patients A, B, C, and D to extracts of iris tissue from the enucleated eyes of Patients C and D.

The MIF test was performed toward extracts of choroidal MM from the enucleated eyes of Patients A, B, C, and D and to extracts of iris tissue from the enucleated eyes of Patients C and D.

choroidal MM in his single eye and is therefore only being kept under close follow-up.

2. Ten patients with choroidal nevus.

3. Five patients with generalized carcinoma.

4. Ten healthy controls.

**Antigens.** Sterile melanoma tissue was obtained from the enucleated eyes of Patients A, B, C, and D. In the enucleated eyes of Patients C and D, a specimen of iris tissue was also taken. Each tissue specimen was minced separately in phosphate-buffered saline and homogenized for 5 to 7 min at 5°C. The protein content of the suspension was determined by the Lowry method. Concentrations of 25, 50, 100, and 200 µg of protein per milliliter of medium were used in the MIF test. It was found that 50 µg/ml protein was the minimal concentration which did not effect the normal migration of macrophages alone and at the same time sensitized the lymphocytes of the MM patients to produce sufficient MIF to inhibit macrophage migration. This was therefore chosen as the optimal concentration to be used in our technique. All extracts were stored at —20°C until used.

**Lymphocytes.** A 20 ml amount of venous blood was drawn from every patient except Patient B, who served only as donor of the tumoral antigenic extract. In the group of patients with uveal MM, the blood samples were drawn prior to enucleation in Patients A, C, D, F, and I; 4 months after enucleation in Patient E; and 6 months after cyclectomy-iridectomy in Patients G and H. Blood specimens were sent for analysis labeled so that neither the name nor diagnosis was known by the investigator. Lymphocytes were separated from whole blood by Ficoll-Isopaque density-gradient centrifugation.

**Macrophages.** The macrophages for the MIF test were obtained from the peritoneal cavity of normal guinea pigs previously injected with paraffin oil. These were washed in phosphate-buffered saline (PBS) and brought to a concentration of 8 × 10⁷ cells/ml of M-199 Hanks’ solution containing 15% heated fetal calf serum (FCS).

**MIF test.** The MIF test was performed according to a modification ¹ of the technique of Rajapakse and Glynn. Results were obtained from the mean migration areas of duplicates with a maximal discrepancy of 15% between them. The migration index (MI) was calculated according to the following formula:

\[
MI = \frac{\text{Area of migration of macrophages + lymphocytes + antigen}}{\text{Area of migration of macrophages + lymphocytes - antigen}} + \left[ 1 - \frac{\text{Area of migration of macrophages + antigen}}{\text{Area of migration of macrophages - antigen}} \right]
\]

**Results.** Table I presents the results of the MIF tests obtained with lymphocytes from eight choroidal MM patients toward two to four choroidal melanoma extracts as well as the histological findings. The mean MI of this group to all four antigens was 0.79 ± 0.20. The mean MI of the 10 normal donors to the MM antigens was 1.02 ± 0.10. The difference between the mean MI of the MM patients and that of the healthy donors was found to be highly significant (p = 0.001). The value of 0.80 or less was therefore considered to be a significant index of migration inhibition (i.e., a positive MIF test).
It was further found that seven of the eight patients had a positive MIF test toward at least one melanoma extract and in most cases toward two or three. The three patients who were tested against their own tumor extracts (A, C, and D) responded positively. One patient (E) failed to respond to any of the three extracts used.

The mean MI of the patients with choroidal nevus toward MM antigens was 1.01 ± 0.05; that is, there was no actual difference between these results and those obtained in the normal donors. None of the patients suffering from generalized carcinomatosis responded to the tumoral extracts of MM. None of the subjects tested in this investigation responded to the iris extracts.

Discussion. Recently Char and co-workers obtained positive skin reactions and inhibition of leukocyte migration in a considerable number of patients with choroidal MM, using as antigen extracts of human skin MM or of liver metastases from cases of choroidal MM. Since the antigenicity of skin and choroidal MM may differ, it was thought that the use of extracts of primary choroidal MM might further improve the specificity of the results obtained.

The fact that the lymphocytes of all the patients who were tested in this study against their own tumor extracts responded with a positive MIF test but the lymphocytes of these same patients showed a variable cross-reactivity in the presence of allogeneic tumor extracts strengthens the possibility of a multiantigenic character of these tumors, with variable expression of the different antigenic components. The fact that in this study there were a greater number of positive results in the tests toward extracts A and D than toward extract C suggests that some MM-associated antigens have a higher frequency than others. In the small group of cases in our study, the antigenic specificity of the tumor extracts appeared to have no relation to the histologic type of tumor. Thus Patient A with spindle cell MM responded to extracts of both spindle and epitheloid MM, whereas lymphocytes from Patient H with epitheloid melanoma responded to spindle-type MM extracts but not to the extract prepared from an epitheloid-type MM. The single patient who showed no response to any of three MM extracts was tested 4 months after enucleation, and it is tempting to speculate that in the interval the sensitization of his lymphocytes toward the-tumoral antigens reversed from positive to negative. Since this patient was not tested against his own tumor extracts, however, it cannot be excluded that his lymphocytes were sensitized against some of the antigens characteristic of his own tumor but not present in the extracts of the other tumors used in this investigation.

None of the patients who responded with a positive MIF test to the choroidal MM extracts did so to extracts of iris tissue taken from the same enucleated eyes. This finding shows that sensitization to the extracts of MM was indeed specific to the tumoral antigens and not to the antigens of normal uveal tissue from the eye bearing the choroidal tumor.

None of the patients with choroidal nevus or carcinomatosis showed a positive MIF test to any of the MM extracts. Should this be further confirmed in a larger group of patients, it could be a real help in the differential diagnosis between primary choroidal MM, choroidal nevus, and metastatic choroidal carcinoma.

It is of particular note that the four patients who were tested prior to enucleation (Patients A, C, D, and F) had positive MIF tests toward tumoral extracts of MM. In all four cases the postoperative histological diagnosis was MM. This finding stresses the diagnostic value of the MIF test performed prior to enucleation, provided that a wide panel of choroidal MM extracts is used to cover the maximal number of antigenic components in order to increase the chances that the patient's lymphocytes will be exposed to the antigens contained in his own tumor.

The number of MM cases available for the present study was too small to allow us to draw definite conclusions. The results obtained, however, would seem to justify further investigation of the possible value of immunological assays in such cases. Since every year there are approximately six cases of choroidal MM among every million persons and since a single tumoral extract can provide the material for a considerable number of MIF tests, it should be possible to build up a national bank of tumoral extracts which would enable us to perform MIF tests toward a battery of antigens in difficult diagnostic cases.

Appendix. Since this paper was written, Patient F (who before enucleation had strongly positive MIF tests against three choroidal allogeneic extracts) was tested against his own tumoral extract. One month after enucleation the MIF test was positive (MI, 0.56), whereas now, 5 months after enucleation, it has become negative (MI, 1.0). This finding supports our opinion that in Patient E the MIF tests against three allogeneic tumoral extracts performed 4 months after enucleation were perhaps negative only because of the time lapse following enucleation.
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Key words: cell-mediated immunity, malignant melanoma, migration-inhibition factor, extracts of choroidal malignant melanoma-associated antigens

REFERENCES

Photosensitive cataractogens, chlorpromazine and methoxypsoralen, cause DNA repair synthesis in lens epithelial cells.

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Autoradiographic techniques showed that photoactivation of methoxypsoralen or chlorpromazine caused diffuse nuclear labeling of lens epithelial cells by thymidine. Chlorpromazine and light also induced thymidine incorporation into lymphocytes, whereas light or drug alone did not cause unscheduled DNA synthesis. Certain photosensitizing agents have been implicated in the production of cataracts in guinea pigs, mice, and humans. 1-4 It has been suggested that photo-induced protein coagulation accounts for the opacification. Of course few, if any, drugs show absolute specificity for a specific cellular component, and many photosensitizers affect DNA as well as protein. 4 Several cataractogenic agents such as x-ray, ultraviolet (UV) radiation, and alkylating agents affect DNA as their major biological target, suggesting the importance of examining lens DNA as a potential site of action of cataractogenic photosensitizers.

We report here that each of two cataractogenic photosensitizers, methoxypsonalen (8-MOP) and chlorpromazine, in combination with near-UV radiation induce unscheduled DNA synthesis ("repair synthesis") in lens epithelial cells. This was observed autoradiographically and is presumptive evidence for repair of damage to DNA. 5 Photosensitized damage to DNA was substantiated by the additional finding that combined treatment with the cataractogens and near-UV radiation provoked repair synthesis in human peripheral lymphocytes treated with hydroxyurea. These results therefore suggest alteration of epithelial cell DNA as an additional or alternate mechanism by which such compounds can produce lens opacities.

Methods

Incubation and irradiation of lenses. Male Osborne-Mendel rats approximately 175 to 200 gm in weight were anesthetized with chloroform, and the lenses were removed. The lenses were then placed in 4 ml of Kinoshita's medium as described by von Sallmann and Grimes, 6 to which the photosensitizer was added. After being incubated with the photosensitizing drug for 1/2 hr at 37°C, the lenses were irradiated cell side up for time periods of 2 to 30 min with a Raymaster lamp (tube type B, Black Raymaster; George W. Gates & Co., Inc., Franklin Square, N. Y.) with its energy peak near 360 nm and practically no radiation shorter than 300 nm, filtered to remove all but a trace of visible light (manufacturer's specifications). This lamp was calibrated with a Yellow Springs Instrument Co. (Yellow Springs, Ohio) meter which recorded a flux of 1.1 × 105 ergs/cm sec at 13 cm, the working distance which was used throughout. A piece of plate glass 0.5 cm thick was placed between the lens and the light source as a means of eliminating extraneous far-UV radiation.

Two types of control lenses were always maintained. One group was kept in the dark after a 1/2