Experimental Autoimmune Anterior Uveitis

The Preparation of Uveitogenic Ocular Melanin

René M. Broekhuyse and Eleonoor D. Kuhlmann

Purpose. The purpose of this study was to develop a rapid procedure for the preparation of melanin with a specific, highly uveitogenic activity.

Methods. A crude melanosome fraction was isolated from bovine choroids (containing remnants of adhering retinal pigment epithelium). The fraction was extracted with hot 2% sodium dodecyl sulfate, and Lewis rats were immunized with the purified melanin, using pertussis toxin as coadjuvant.

Results. The purified melanin was free from pathogenic photoreceptor antigens and other accompanying or adsorbed proteins. It was able to evoke severe, acute, anterior uveitis with the typical characteristics of experimental autoimmune anterior uveitis (EAAU), even at the level of 1 µg melanin protein.

Conclusions. The rapidly prepared ocular melanin exhibits the same qualities as purified choroidal or retinal pigment melanins obtained by much more laborious procedures (which also deliver other subcellular fractions for investigation). It is suitable for the study of the immunopathogenesis of EAAU, which is a new model for human acute anterior uveitis. Invest Ophthalmol Vis Sci. 1993;34:698–700.

From the Institute of Ophthalmology, University of Nijmegen, Nijmegen, The Netherlands.

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Reprint requests: Dr. R. M. Broekhuyse, Institute of Ophthalmology, University of Nijmegen, Philips van Leydenlaan 15, 6500 HB Nijmegen, The Netherlands.
MELANIN PREPARATION

Eyes

Eye cups

rinses (PBS)

Choroids (+ RPE)

crush, sieve, wash

Crude melanosomes

hot 2% SDS

Chor(m)SI-r

FIGURE 1. Melanin preparation.

preparations were characterized by melanin protein determination,2 granule counting,3 dry weight determination,3 SDS-polyacrylamide gel electrophoresis, and electroimmunoblotting1 of an SDS extract (5% SDS–0.1 mol/L dithiothreitol [DTT], 20 μL/50 μg melanin protein, 3 hr at room temperature or 10 min at 80°C), and by immunization of rabbits (for testing of immunologic reactivities)12 and of Lewis rats for the study of the uveitogenicity.

Lewis rats were injected in each hind foot pad with 0.1 ml emulsion of an antigen suspension in complete Freund’s adjuvant (Difco Laboratories, Detroit, MI), or with 0.03 ml emulsion in Hunter’s adjuvant (CytRx Corp., Norcross, GA). Half the antigen dose was injected in the foot pads, and the other half was mixed with a solution of 1 μg pertussis toxin (Sigma, St. Louis, MO) in PBS and injected intraperitoneally.1–3 The eyes were examined clinically and histologically.12 The animal experiments conformed to the ARVO Resolution on the Use of Animals in Research.

RESULTS. The described method enabled the preparation of purified choroidal melanin granules from bovine eyes within a few hours. Per eye, 3.43 ± 0.01 mg (n = 3) of melanin protein (2.6 × 109 granules/mg) was obtained (the recovery of the RPE melamin protein, prepared as described in previous reports,1,2 amounted to 200 μg per eye, or 6% of the choroidal melanin protein). The protein:dry weight ratio amounted to 1.25 ± 0.1 (n = 5). On an SDS-polyacrylamide gel electropherogram of an SDS-DTT extract of Chor(m)SI-r, no polypeptides could be found, showing that no adsorbed protein was present any more. Electroimmunoblots of such electropherograms, reacted with rabbit antisera versus bovine Chor(m)SI-r, bovine S-antigen, interphotoreceptor retinal binding protein (IRBP), and rhodopsin, did not reveal any antigenic polypeptide either, in agreement with previous studies of SDS-extracted melamin.2,3

Lewis rats immunized with bovine Chor(m)SI-r in complete Freund’s adjuvant and pertussis toxin contracted severe EAAU (Table 1). At a dose of 5 μg, the inflammation started at day 10; at low doses a considerable delay in the onset occurred, but even doses of 1 or 2 μg were able to evoke severe EAAU in some of the animals (score 4). With Hunter’s adjuvant, a more severe reaction was evoked with an earlier onset, and the foot pads were less inflamed (Table 1). On repetition of the experiments, virtually the same results were obtained. The characteristics of the disease were identical to those described previously.1–3 Briefly, they consisted of acute severe anterior uveitis often followed by mild choroiditis. Retinitis and pinealitis were absent. EAAU started to regress after 1 or 2 wk after its onset. After 2 mo, the only ocular abnormalities were dystrophic epithelial cells on iris and ciliary body, and an occasional swollen focus in the iris.

DISCUSSION. The described method for the preparation of uveitogenic ocular melanin is based on a series of previous studies.1–3 The following considerations and data led to this procedure. It appeared that buffer, Triton X-100, and SDS-extracted melamin

<table>
<thead>
<tr>
<th>Injected Antigen</th>
<th>Adjuvant</th>
<th>Dose (μg)</th>
<th>Incidence</th>
<th>Mild</th>
<th>Severe</th>
<th>Maximum Score†</th>
<th>Day of Onset†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chor(m)SI-r</td>
<td>CFA</td>
<td>5</td>
<td>4/4</td>
<td>0</td>
<td>4</td>
<td>4.0 ± 0.0</td>
<td>10.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5/5</td>
<td>1</td>
<td>4</td>
<td>3.1 ± 1.1</td>
<td>16.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1/4</td>
<td>0</td>
<td>1</td>
<td>1.0 ± 1.3</td>
<td>23</td>
</tr>
<tr>
<td>Chor(m)SI-r</td>
<td>HA</td>
<td>2</td>
<td>5/5</td>
<td>0</td>
<td>5</td>
<td>4.0 ± 0.0</td>
<td>10.4 ± 0.5</td>
</tr>
</tbody>
</table>

* Complete Freund’s adjuvant (CFA) or Hunter’s adjuvant (HA) was used.
† Mean values (± SEM) of the maximum scores of the group.
from RPE and choroid are immunologically related, are equally uveitogenic, and evoke an identical inflammatory reaction. If the aim of a study is merely to make use of the EAAU model, and it is not necessary to prepare these melanins separately, the tissue isolation techniques can be speeded up considerably by leaving the RPE on the choroid. Furthermore, it appeared that the direct extraction of the crude melanin with hot 2% SDS abrogates the need for preceding buffer and Triton X-100 extractions. If the products of the latter two extracts are not needed for investigation, the hot SDS treatment can be applied directly.

In pilot studies, we found that temperature elevation during SDS extraction increases the effectiveness of protein dissolution without influencing the pathogenicity of the melanin. It effectively removes all adherent protein (including the melanosomal membrane), S-antigen, IRBP, and (rhod)opsin. The resulting preparation technique for melanin antigen is the most simple method among the isolation techniques of uveitogenic ocular antigens available to date because no chromatographic or adsorption procedures are required. The obtained product is highly stable and pathogenic. By immunization with 5 μg of bovine ocular melanin protein, a 100% incidence of EAAU is obtained. The clinical and histologic details are identical to those reported for purified melanins from bovine RPE and choroid that contain the (probably identical) hypothetical antigens PEP-X and UP-X, respectively.1-3 Hence, the described isolation procedure adequately delivers a technically well defined melanin antigen for induction of the new model for anterior uveitis, EAAU, and also is in principle applicable to the preparation of ocular melamins of other species, including humans.3

The finding that ocular and skin melamins are potential pathogens is of great interest for the study of pigment-related diseases of the eye and other tissues.3 This melanin-associated activity awaits further characterization and localization.

Key Words
choroid, experimental autoimmune anterior uveitis, melanin preparation, retinal pigment epithelium

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References