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LETTER TO THE EDITORS

Intraperitoneally Injected Melanin is Highly Uveitogenic

Experimental melanin-protein induced uveitis (EMIU; also called experimental autoimmune anterior uveitis, EAAU) is usually evoked by foot pad immunization of Lewis rats with ocular melanin, in some protocols supported by intraperitoneal injection of additional antigen and pertussis toxin (Broekhuyse et al., 1992a; Chan et al., 1994). EMIU in rats is clinically first recognized as severe anterior uveitis. Choroiditis also develops, and on the long term the disease tends to exhibit multiple spontaneous recurrences (Broekhuyse et al., 1995). The foot pad immunization protocol is used to evoke several types of experimental autoimmune diseases. It has the drawback of the need for Freund's complete adjuvant or Hunter's adjuvant which cause inflammation of the hint foot pads. Various alternatives have been proposed for the avoidance of the use of harmful adjuvants (Claassen and Boersma, 1992; review). In addition, the search for mild protocols reveals the variable pathogenicities of the antigens in rats. They depend on the applied, specific immunization techniques as appears from the latter review, and from the present report. We show that EMIU can efficaciously be induced merely by the intraperitoneal injection of melanin. This method appears to be less successful in the induction of uveitis if specific retinal autoantigens are used.

Purified melanins were isolated from bovine iris and choroid as described previously (Broekhuyse et al., 1993a; 1993b). Briefly, the pigment granules were freed from tissue and tissue debris by homogenization, filtering and sedimentation. They were purified by extraction with 2% sodium dodecyl sulphate (SDS) at 75°C for 10 min, and the resulting SDS-insoluble (SI) fraction was washed with water. The obtained melanin preparations [Iris(m)SI and Chor(m)SI, respectively] were stored at −25°C. The photoreceptor antigens S-antigen and interphotoreceptor retinoid binding protein (IRBP), and the (unpigmented) Triton X-100 soluble bovine retinal pigment epithelial membrane protein (RPE-TS) were prepared from bovine neuro-retina and RPE cells, respectively. Protein determinations, controls for purity by SDS-gel polyacrylamide gel electrophoresis, immunoblotting, and clinical assessment of uveitis were carried out according to Broekhuyse et al. (1986; 1992a; 1992b).

The purified (shortly sonicated) antigens together with 1 or 2 μg pertussis toxin (Sigma, St. Louis, MO, U.S.A.) were intraperitoneally injected in 1·0 ml phosphate buffered salt solution into female Lewis rats of 150–180 g weight. Daily clinically assessment of uveitis started at day 8. EMIU induced by the melanins, experimental autoimmune pigment epithelial protein-induced uveitis (EAPU), and experimental autoimmune uveitis (EAU) evoked by photoreceptor antigens were scored on a scale of 0–4 as described in the respective literature cited above. The experiments were terminated at a score of 4, or otherwise within 1 week after a maximum score was attained. The eyes were fixed in Bouin solution, and processed according to standard histological techniques.

Table I shows that intraperitoneally injected choroidal and iris melanins are highly pathogenic. All animals developed severe EMIU despite the omission of foot pad immunization with the use of Freund's or Hunter's adjuvant. Coinjection of pertussis toxin is a prerequisite for the development of uveitis. Clinically and histologically, the disease appears identical to EMIU evoked by foot pad immunization with the same dose. The photoreceptor antigens S-antigen and IRBP, and the RPE antigen RPE-TS exhibit low pathogenicity via the intraperitoneal route. High doses (70–200 μg) induce uveitis with a low to moderate incidence, and a mostly moderate intensity. Also in these cases, the inflammatory reactions appear very similar to those evoked by the foot pad immunizations.

In contrast to the latter results, it has been shown that foot pad immunization of Lewis rats with 40–50 μg doses S-antigen or IRBP in Freund's adjuvant even without the use of pertussis toxin efficaciously induces EAU. Together with 1 μg pertussis toxin a dose of only 16 μg or less of the photoreceptor antigens evokes severe EAU with 100% incidence (Gery et al., 1986; Broekhuyse et al., 1986). Similarly, immunization with 75 μg RPE-TS in the foot pads and in addition with 75 μg intraperitoneally induces severe EAPU in all injected rats (Broekhuyse et al., 1992b). Hence, the results in Table I show that ocular melanins have a marked capacity to evoke severe uveitis with high incidence after intraperitoneal injection even at low doses. This efficient immunization might be ascribed to the granular character of the material and the specific location of the pathogen. The uveal melanin granules are usually ball and egg shaped with dimensions between 0·2–1·2 μm, and are coated with the melano-antigen UP-X. They are phagocytized by (peritoneal) macrophages which digest this surface pathogenicity. If the surface protein is completely removed the granules are no longer pathogenic (Broekhuyse et al., 1993b, 1993c). In the immunization protocol described above, melanin granules seem to represent the UP-X antigen's own adjuvant, and the protocol thus provides a mild

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TABLE I

Uveitis evoked in Lewis rats by intraperitoneal antigen injection

Antigen	Antigen dose (μg)	Ptx* dose (μg)	Eyes with uveitis†			Maximum score‡	Day of onset‡
			Incidence	Mild	Severe		
Chor(m)SI	5	1	6/6	0	6	4·0±0·0	13·0±1·2
Iris(m)SI	5	1	4/4	0	4	4·0±0·0	11·5±1·2
Iris(m)SI	25	0	0/4	0	0	0·0	—
S-antigen	70	1	1/6	0	1	3	10
IRBP	70	1	1/6	0	1	4	25
IRBP	150	2	4/6	2	2	3·0±0·6	14·0±2·3
RPE-TS	200	2	4/6	2	2	2·5±0·9	11·3±0·6

* Ptx, pertussis toxin.
† Mild, score 1 and 2; severe, score 3 and 4.
‡ Mean values±S.E.M.

technique to evoke uveitis in rats. The results may also have some implications for our understanding of the uveitogenesis in humans. Involvement of photoreceptor antigens in human autoimmune uveitis has repeatedly been reported (Kijlstra et al., 1990), whereas the role of melanin(-protein) in autoimmune disease is presumed (Broekhuyse et al., 1992a; Chan et al., 1994). The present results make it more likely that melanin(-protein) per se is capable of evoking autoimmune responses as well.

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