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Protective effect of pristane on experimental autoimmune uveitis

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ABSTRACT

This study evaluates the effects of pristane and phytol, two mineral oils with pro-oxidative effects, on the course of experimental autoimmune uveitis.

C57BL6 mice were immunized with IRBP1–20 peptide emulsified in CFA and treated five days prior to immunization with phytol or with pristane or with PBS as control.

Administration of pristane reduces the incidence and severity of IRBP-induced uveitis as demonstrated by the decrease in vasculitis and inflammatory foci in fundus and by a reduction in histological damages and leukocyte infiltration compared to untreated or phytol-treated mice. The protective effect observed is associated with a decreased activation of peripheral CD4+ and CD8+ T lymphocytes and a decrease in the intensity of the Th1 and Th17 autoimmune response to IRBP in pristane-treated mice compared to control mice, as evidenced by the decreased production of IFNγ and IL17 by IRBP-specific lymphocytes from lymph nodes draining the site of immunization and by the increased production of anti-IRBP IgG1 over IgG2a. In addition, HUVEC and ARPE-19 cells incubated with the sera of mice treated with pristane presented a reduced production of H_2O_2. The benefit of lowering the systemic oxidative stress by pristane in the course of EAU was confirmed by injecting the antioxidant NAC in IRBP-immunized mice. As pristane, NAC decreased clinical and histological inflammation of the retina and preserved the integrity of the hemato-retinal barrier.

Finally, the protective effect of pristane on the development of EAU suggests that some mineral oils may represent a new therapeutic strategy in human uveitis.

1. Introduction

Experimental autoimmune uveoretinitis [1] has been studied as an animal model of birdshot retinochoroidopathy, sympathetic ophthalmia, Vogt-Koyanagi-Harada's disease or Behçet's disease. EAU is an organ-specific, T cell-mediated autoimmune disease that can be induced by immunization with retinal Ag [2,3], e.g., human interphotoreceptor retinoid-binding protein (IRBP) or by the adoptive transfer of retinal Ag-specific T lymphocytes [4]. EAU is the consequence of a Th1/Th17 dominant response [5]. Augmentation of the Th2 response and T regulatory cytokine production or down-regulation of the Th1/Th17 response prevents inflammatory responses and prevents the development of EAU [6].

In human, uveitis is often associated with systemic inflammatory diseases such as Behçet disease, spondylarthritis or sarcoidosis. A chronic oxidative stress caused by an imbalance between pro- and anti-oxidant molecules seems to be a common feature of the chronic inflammatory state observed in those diseases. However, a correlation between the intensity of the oxidative stress and the occurrence of uveitis has been described only in Behçet's disease [7].

Superoxide radicals are involved in the pathogenesis of experimental endotoxin-induced uveitis since their detoxification by the antioxidant molecule ebselen prevents the disease in both rabbits and rats [8,9]. Similarly, *in vivo* inhibition of iNOS by L-NAME prevents EAU in Lewis rats by abrogating photoreceptor apoptosis, but L-NAME spares infiltrating CD3 and CD4T cells by compromising their functional inactivation [10]. Paradoxically, the oral administration of the NO-donor GSNO significantly decreases autoreactive T cell activation and diminishes the levels of inflammatory

Abbreviations: EAU, experimental autoimmune uveoretinitis; IRBP, interphotoreceptor retinoid-binding protein; IL, interleukine; IFN, interferon; ip, intraperitoneal; NAC, N-acetylcysteine; sc, subcutaneous.

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mediators in the retina of EAU mice, thus preserving normal retinal histology and function. Altogether, these observations have led to the new hypothesis that ROS and/or RNS could be protective rather than deleterious in certain types of uveitis.

Recent observations in animal models of arthritis are in line with this hypothesis. Indeed, Ncf1, a gene locus whose activation decreases the intensity of the oxidative burst, is a locus of susceptibility to arthritis in murine models [11]. Accordingly, phytol increases the oxidative burst in DA rats, susceptible to arthritis, decreases the autoimmune T cell response and ameliorates both acute and chronic phases of arthritis. By contrast, pristane, another mineral oil structurally close to phytol, induces arthritis in this model [12].

We have evaluated the effects of pristane and phytol on the course of EAU a T-cell mediated autoimmune disease. We have demonstrated that pristane prevents EAU by dampening the Th1/Th17 autoimmune response, by inducing a protective Th2 autoimmune response and by diminishing the permeability of the blood-ocular barrier. By contrast, phytol has no effect on EAU.

2. Materials and methods

2.1. Reagents

Human interphotoreceptor binding protein (IRBP1–20; SGIPYI-ISYLHPGNTILHVD) was purchased from Neosystem (Strasbourg, France), Complete Freund's Adjuvant (CFA) from Sigma–Aldrich (Saint-Quentin Fallavier, France) and *Mycobacterium tuberculosis* strainH37RA from Thomas Scientific. *Bordetella pertussis toxin* (PTX), phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), pristane (2,6,10,14-tetramethylpentadecane) and all other chemicals were from Sigma–Aldrich (Saint-Quentin Fallavier, France).

2.2. Animals, immunization and treatment

Female C57BL6 mice (6–7 weeks old) were purchased from Harlan (Ganna, France) and maintained with food and tap water *ad libitum*. All mice were housed in autoclaved cages with free access to food and water. They were given humane care according to the guidelines of our institution and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice were injected intraperitoneally (i.p.) in the hind legs and the base of the tail with 150 μg of IRBP1–20 peptide (Neosystem, NeoMPS, Strasbourg, France) emulsified (1:1) with CFA (containing 1.5 mg/ml of *M. tuberculosis*) in a total volume of 100 μl. At the same time, an intraperitoneal (i.p.) injection of 1 μg of PTX was performed. Five days before immunization, mice were injected i.p. with either 350 μl phytol ($n = 12$) or 350 μl pristane ($n = 10$) or PBS as control ($n = 14$). Treatment with N-acetyl cysteine (Bristol-Myers Squibb – Rueil Malmaison – France) was administered 3 days a week at a dosage of 150 mg/kg also starting 5 days prior to immunization until the day of sacrifice (23 days after immunization).

2.3. Cell culture

The Human Retinal Pigment Epithelial Cell Line ARPE-19 was grown in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen Ltd., Paisley, UK Ltd., Paisley, UK) with GlutaMax and supplemented with 10% fetal bovine serum (FBS, Invitrogen Ltd., Paisley, UK), 10 mM Hepes, 1 mM sodium pyruvate, and antibiotic/antimycotic solution (10 U/ml pencillin, 10 μg/ml streptomycin, 0.25 μg/ml amphotericin) (Invitrogen Ltd., Paisley, UK) at 37 °C in humidified atmosphere containing 5% CO_2. The cell line was routinely tested for *Mycoplasma* infection.

Human umbilical vein endothelial cells (HUVEC) were obtained by digestion of umbilical cords with 0.1% collagenase and cultured as previously described [13]. HUVEC were not used beyond the third passage.

2.4. Clinical evaluation of EAU and scoring

Clinical assessment by funduscopic examination of the retinal inflammation was conducted every other day from day 13 after immunization. The severity of EAU was graded from 0 to 4, as described previously [14]. Briefly, the clinical scoring was based on vessel dilatation, the number of vascular white focal lesions, vascular white linear lesions, haemorrhages, and the extent of retinal detachment.

2.5. Histopathological evaluation of EAU and scoring

Clinical EAU was evaluated by periodic fundus examination and was confirmed by histological examination. For the histological assessment of EAU, the eyes were collected 23 days after immunization, within 1 min of euthanasia. The eyes were prefixed for 1 h in 4% phosphate-buffered glutaraldehyde, then transferred into 10% phosphate-buffered formaldehyde until processing. Fixed samples were embedded in paraffin, and 5-μm sagittal sections were cut near the head of the optic nerve and stained with hematoxylin and eosin. The severity of EAU was scored on a scale from 0 to 4 as described previously [14]. Briefly, focal nongranulomatous monocytic infiltrates in choroid, ciliary body, and retina were scored as 0.5. Retinal perivascular infiltrates and monocytic infiltration in the vitreous were scored as 1. Granulomatous formations in the uvea and retina, associated with occluded retinal vasculitis, photoreceptor folds, serous detachment and loss of photoreceptors, were scored as 2. In addition, the formation of Dalen-Fuchs nodules (granulomatous lesions in the retinal pigmented epithelium) and development of subretinal neovascularization were scored as 3 and 4, according to the number and the size of the lesions. EAU severity was double-blindly scored by two ophthalmologists.

2.6. Determination of immunological responses

IRBP-specific Delayed Type Hypersensitivity (DTH) was determined by an ear swelling assay 12 days following immunization as described previously [15]. Briefly, 10 μg of IRBP peptide in 10 μl PBS were injected into the right ear pinna, and 10 μl PBS alone were injected into the left ear pinna. After 48 h, the antigen-specific response was calculated by determining the difference in thickness (μm) between the antigen- and the PBS-injected ear using a micrometer calliper.

For the lymphocyte proliferation assay, spleens were collected on the day of sacrifice. Splenocytes (2×10^5) in 200 μl complete media were cultured with or without 10 μg/ml IRBP peptide, for 72 h. Cell proliferation was measured in triplicate by pulsing the cells with [^{3}H]thymidine (1 μCi/well) during the last 18 h of culture. Results were expressed as absolute numbers of counts per minute (cpm). For IRBP-specific production of cytokines by lymphocytes in primary cultures, inguinal and iliac lymph nodes were collected in each group at the end of the experiment and pooled within each group. Cytokines were quantified in 48-h supernatants of IRBP-stimulated cells using standard ELISA kits for IFN-γ and IL-4 (OptiEIA; Pharmingen, BD Biosciences, Le Pont de Claix, France) and for IL17 (Mouse IL-17 Quantikine ELISA Kit, R&D, Lille, France). Each immunoassay is calibrated against purified recombinant mouse IL-4 or IL17 supplied with the kit. The blood drawn at the sacrifice was tested for antinuclear antibodies, rheumatoid factors and anti-dsDNA antibodies. All the results were negative in each mouse.