Interleukin 10 inhibits inflammatory cells infiltration in endotoxin-induced uveitis

Received: 3 January 1996
Revised version received: 3 May 1996
Accepted: 11 June 1996

Seiji Hayashi
Yan Guex-Crosier
Anne Delvaux
Thierry Velu
François G. Roberge

S. Hayashi • Y. Guex-Crosier
F.G. Roberge (~)
National Eye Institute,
National Institutes of Health,
Bldg. 10, Room 10N 202,
10 Center Drive, Bethesda,
MD 20892-1858, USA

A. Delvaux • T. Velu
Institut de Recherche Interdisciplinaire
and Department of Medical Genetics,
Erasme Hospital,
Free University of Brussels,
808 route de Lennik, B-1070 Brussels,
Belgium

Abstract  • Background: Endotoxin-induced uveitis (EIU) is a model for acute anterior uveitis associated with a variety of pro-inflammatory cytokines and nitric oxide production. Interleukin 10 (IL-10) down-regulates these inflammatory mediators. We report a study of the effect of systemic administration of IL-10 on the inflammatory parameters of EIU. • Methods: Uveitis was induced in C3H/HeN mice by subcutaneous injection of 200 µg lipopolysaccharide (LPS) per mouse. Intraocular inflammation was assessed by leukocyte count and measurement of the protein concentration in the aqueous humor (AH). Mouse recombinant IL-10 at 1000 U or its vehicle alone were administered by three intravenous injections given 4.0 h and 0.5 h before and 8.0 h after LPS injection. • Results: The inflammatory cell infiltration in the eyes was significantly reduced in four of five experiments from 40% to 64% in the groups treated with IL-10 compared to the control groups (P<0.05). In contrast, the level of protein exudation in the anterior chamber (AC) was not significantly affected by IL-10 treatment. • Conclusion: IL-10 reduces the cellular infiltration in the ocular inflammation produced by endotoxin. This result suggests potential usefulness for IL-10 in the treatment of severe anterior uveitis with a strong cellular component.

Introduction

Interleukin 10 (IL-10) is a 17- to 21-kDa protein secreted by activated TH2 lymphocytes, macrophages, Ly-1 B cells, and keratinocytes [review: 15] It was originally identified as an inhibitor of the TH1 cell population responsible for delayed-type hypersensitivity reactions [6]. Subsequently, IL-10 was found to have pleiotropic effects on several cells of the immune system. A major aspect of these effects is a down-regulatory action on the mediators of the inflammation caused by bacterial endotoxins [7, 10]. In particular, IL-10 inhibits the synthesis of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), IL-1, and IL-6, all of which have been implicated in the cardiovascular shock caused by endotoxin [1, 14, 21]. As a consequence of this deactivating effect on macrophages, the production of nitric oxide is also inhibited [2, 3]. The temporal sequence of the secretion of cytokines is important for their coordinated effects. In the course of the response to endotoxin, the release of IL-10 closely accompanies the secretion of pro-inflammatory cytokines [5, 14]. There is evidence that IL-10 then acts to suppress further production of these cytokines [1, 14, 21]. This observation suggested that IL-10 might prevent the noxious effect of endotoxin if given early enough. Indeed the injection of IL-10, before or at the same time as lipopolysaccharide (LPS), reduced the mortality from cardiovascular shock [7, 10]. An extensive number of inflammatory cytokines have also been found associated with the development of the uveitis produced by endotoxin [17, 20, 23]. In addition, we and others have shown that nitric oxide is crucial in the induction of endotoxin-
induced uveitis (EIU) [13, 16, 19]. However, a certain amount of controversy remains as to the role played by these vaso-collapsing factors in the pathogenesis of the ocular disease. We are reporting here a first series of experiments examining the possible similarity in the mechanism of control of the uveitis and the vascular response to endotoxin. Specifically, we tested the influence that systemic IL-10 administration could have on the course of the ocular inflammation in the EIU mouse model. In this model, a subcutaneous injection of LPS to C3H/HeN mice causes a transient fibrinoid exudation in the AC, accompanied by an infiltration with polymorphonuclear neutrophils (PMN) and macrophages [12]. The reaction peaks around 24 h after LPS injection and last between 2 and 3 days.

**Materials and methods**

Induction of uveitis and IL-10 administration protocol

Female C3H/HeN mice (Charles River, Raleigh, N.C.), 6–8 weeks old and weighing 18–20 g, were injected in one hind footpad with 200 μg of LPS from *Salmonella typhimurium* (Difco, Detroit, Mich.) in 50 μl of phosphate-buffered saline solution pH 7.2 (PBS). Mouse recombinant IL-10 was produced by the SF9 cell line through a baculovirus vector containing the mouse IL-10 eDNA, as described by Delvaux and colleagues [4]. IL-10 at 1000 U in 0.3 ml PBS, or PBS alone, were administered three times via intravenous injection in the tail vein at 4.0 and 0.5 h before and 8.0 h after LPS injection. Five experiments were done with groups of 6–15 mice receiving IL-10, and 6–15 mice receiving PBS alone. In addition 15 naive mice were treated with IL-10 to assess any independent effect on the eye. The principles of laboratory animal care of NIH publication No. 86-23, revised in 1985, were followed.

Disease evaluation

Twenty-four hours after endotoxin injection, the mice were killed by CO₂ inhalation. The AH was collected from both eyes using a 33-gauge needle, and pooled for each animal. One microliter of AH was placed on a silanated glass slide (Digene, Beltsville, Md.) and allowed to dry. The rest of the AH was immediately centrifuged at 10000 g and the cell-free AH was diluted 1:10 in isotonic 5 mM ethylenediamine tetraacetic acid (EDTA). The cells were counted after staining with 0.4% trypan blue solution under a cover slip. The AH protein content was measured using the Coomassie colorimetric assay in reference to a bovine albumin standard (Pierce, Rockford, Ill.).

Statistical analysis

The data were analyzed by Mann-Whitney rank sum test using the StatView 4.5 software (Abacus Concepts, Berkeley, Calif.).

**Results**

In repeated experiments, the intravenous administration of IL-10 significantly reduced the inflammatory cell infiltration in the eye. In five consecutive experiments, the proportional decrease in the number of AH leukocytes was 62%, 64%, 48%, 11%, and 40% respectively. The decrease was statistically significant in four of the five experiments (Fig. 1).

In contrast, IL-10 did not produce a consistent effect on protein exudation in the AC (Fig. 2). The protein levels measured in the AH of the IL-10-treated mice were reduced in one of the experiments, increased in the next, and approximately equal to the levels in the PBS control groups in the following three experiments. The overall average level in the IL-10-treated mice was 3.2 ± 0.2 mg/ml compared to 3.1 ± 0.2 mg/ml in the controls.

The intravenous injection of IL-10 alone in naive mice had no effect on the cellular and protein content of the AH (data not shown).

**Discussion**

Interleukin 10 was very effective at preventing the cellular infiltration of the eyes of mice injected with endotoxin. On the other hand, the absence of reduction in protein leakage was somewhat surprising, since IL-10 inhibits the cytokines that are responsible for vascular dilation.