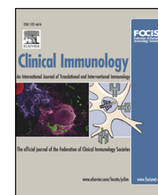
Clinical Immunology 173 (2016) 27–31

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim



BRIEF COMMUNICATION

Phenotypic changes of peripheral blood mononuclear cells upon corticosteroid treatment in idiopathic intermediate uveitis

Karoline Walscheid [a,b,*], Toni Weinhage [b], Dirk Foell [b], Carsten Heinz [a,c], Maren Kasper [a], Arnd Heiligenhaus [a,c]

[a] Department of Ophthalmology, Ophtha Lab at St. Franziskus-Hospital, Muenster, Germany
[b] Department of Pediatric Rheumatology and Immunology, University Hospital Muenster, Germany
[c] Department of Ophthalmology, University of Duisburg-Essen, Germany

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 10 August 2016
accepted with revision 24 October 2016
Available online 29 October 2016

Keywords:
Uveitis
Pathogenesis
Monocytes
T cells

ABSTRACT

We analyzed phenotype and function of peripheral blood mononuclear cells in 9 patients with active idiopathic intermediate uveitis (IIU) before and after 6 and 12 weeks of systemic corticosteroid (CS) treatment and compared to 28 healthy individuals. Monocytes from IIU patients showed increased MHCII expression compared with controls ($p = 0.09$). Treatment reduced expression of MHCII, CD86, CD39 and CD124 (all $p < 0.05$), whereas the percentage of CD121b-expressing monocytes was increased by week 6 ($p = 0.039$). Patients showed alterations in T cell polarization (Th1/Th2 ratio: patients 5.2 versus controls 3.1, $p = 0.054$; Th17/Treg ratio: 3.0 versus 1.7, $p = 0.027$). S100A12 serum levels were higher in active IIU ($p = 0.057$). Phagocytosis, oxidative burst and serum cytokine levels did not differ between patients and controls, and were not altered by treatment. In conclusion, monocytes from patients with active IIU show increased co-stimulatory capacities, which are modulated by systemic CS treatment, whereas innate immune cell functions are not altered.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Intermediate uveitis is a clinical entity accounting for approximately 20% of uveitis cases in adults and children [1–3]. In up to 40%, it is associated with systemic immune-mediated diseases, such as multiple sclerosis or sarcoidosis, or infectious diseases [1,4]. Up to 60% are considered to be idiopathic (namely, idiopathic intermediate uveitis, IIU) [1].

Pathogenesis of IIU remains vastly unclear. Based on data from animal models reflecting inflammation involving the posterior segment of the eye (experimental autoimmune uveoretinitis, EAU), it is considered to be an antigen-specific T cell-mediated disease, with especially Th1 and Th17 cells being involved in initiation and perpetuation of ocular inflammation, and regulatory T cells (Treg) limiting disease [5]. Additionally, monocytes/macrophages are considered to be important effector cells of disease [5–8].

However, data on peripheral blood mononuclear cells (PBMCs) in the clinical setting with patients suffering from IIU are scarce. Several studies report alterations in the peripheral blood T cell compartment regarding phenotype, function or activation status [9–11]. In contrast, only very little is known about peripheral blood monocyte phenotype and function in patients with IIU, despite the importance of interactions between innate and adaptive immune system observed in EAU [12–14].

Locally and systemically applied corticosteroids (CS) are a mainstay in therapeutic concepts of autoimmune uveitis. However, little is known regarding their effects on PBMCs in uveitis patients. One recent study found alterations in monocyte subsets in uveitis patients under systemic CS treatment [12]. In vitro studies of dexamethasone-treated monocytes found an induction of a distinct regulatory monocyte phenotype by CS treatment (glucocorticoid-stimulated monocytes, GCsMs) [15–17].

In this study, we therefore investigated the immune cell phenotypes and functions in peripheral blood from patients with IIU. We describe the effect of systemically administered steroid treatment on PBMCs, with a main focus on the changes in co-stimulatory and regulatory potential of monocytes.

2. Material and methods

2.1. Patients

Uveitis patients were recruited in the Department of Ophthalmology at St. Franziskus-Hospital Muenster. All patients fulfilled the diagnostic criteria of IIU [18]. Patients were diagnosed with idiopathic uveitis after exclusion of associated systemic diseases. For this purpose, patients were assessed by rheumatologists/specialists for internal medicine, neurologists, underwent chest x-ray, cerebral MRI and laboratory testing (differential blood count, CRP, kidney and liver function parameters, electrolytes, urine analysis, angiotensin-converting enzyme,

* Corresponding author at: Department of Ophthalmology, St. Franziskus-Hospital, Hohenzollernring 74, 48145 Muenster, Germany.
E-mail address: karoline.walscheid@uveitis-zentrum.de (K. Walscheid).

http://dx.doi.org/10.1016/j.clim.2016.10.013
1521-6616/© 2016 Elsevier Inc. All rights reserved.

interleukin-2-receptor, Treponema pallidum particle agglutination assay, tuberculosis screening (interferon-γ release assay)).

Patients with active IIU requiring systemic treatment were asked to participate in this study. At baseline, none of the patients received systemic anti-inflammatory medication. In conformity with current treatment recommendations in Germany [1], patients received oral CS starting at 1 mg/kg body weight daily for one week, with subsequent tapering of the dosage during 6 weeks until a maintenance dose of ≤0.1 mg/kg/day was reached. Additional topical steroids were permitted.

Control samples were obtained from healthy volunteers without a history of systemic or ocular immune-mediated diseases, and without any signs of infections during the previous 4 weeks.

Written informed consent of patients/volunteers or patients´ parents (in case of four patients <18 years) was obtained. The study was conducted according to the Declaration of Helsinki, and was approved by the local ethics committee.

2.1.1. Clinical data

Patients underwent ophthalmological examination before (w0), as well as 6 (w6) and 12 (w12) weeks after starting CS therapy. Clinical data were recorded as described previously [19].

2.1.2. Samples

10 ml of peripheral venous blood were collected from each patient at w0, 6 and 12; healthy volunteers donated blood once. Blood for flow cytometry analysis was collected in Lithium-Heparin tubes and processed immediately. Serum samples were centrifuged at 1000 x *g* for 10 min immediately after acquisition, and aliquots were stored at −80 °C until analysis.

2.2. Flow cytometry analysis

2.2.1. Antibodies & reagents

For flow cytometry staining the following antibodies were used: CD80 (Pe;2D10), CD86 (Pe;IT2.2), FoxP3 (AF488;259D), CD4 (APCCy7;RPA-T4), CD8 (PerCP;RPA-T8), CD39 (APC;A1) (all purchased from BioLegend, San Diego, CA, USA), T-bet (PeCy7;eBio4B10), Gata-3 (eFluor 660;TWAJ), RORγt (Pe;AFKJS-9), CD14 (FITC;c61D3), CD16 (APC/Pe;eBioCB16), MHCII (Pe;LN3) (eBioscience, San Diego, USA), CX3CR1 (Pe;528728), CCR2 (Pe;48607), CD121b (Pe;34141) (R&D Systems, Minneapolis, USA), CD124 (Pe;hIL4R-M57) (BD Biosciences, Heidelberg, Germany).

2.2.2. Flow cytometry analysis

For monocyte phenotyping, 100 μl of whole blood were stained for 30 min with antiCD14 and CD16 antibodies, in combination with one of the following: CD121b, CX3CR1, CCR2, CD124, MHCII, CD80, CD86 or CD39. Afterwards, erythrocytes were lysed and cells fixed (using a fix/lysing solution; BD Biosciences) for 10 min at room temperature. Cells were washed twice in PBS (with 2% fetal calf serum, 2 mM EDTA, 0.05% NaN_3) and re-suspended.

For T cell phenotyping, 200 μl of whole blood were stained for CD4 and CD8. Erythrocytes were lysed, cells fixed and permeabilized using a FoxP3/transcription factor staining kit (eBioscience) according to manufacturer's instructions. Intracellular staining was performed for FoxP3, RORγt, GATA3 and T-bet.

Samples were measured using a FACSCanto A flow cytometer (BD Biosciences) and analyzed with FlowJo software version X.0.7 (Tree Star Inc., Ashland, USA). Percentage of positive cells and mean fluorescence intensity (MFI; geometric mean) were documented.

The gating strategy and representative blots for monocytes are depicted in Supplementary Figs. 1 & 6. Cell subsets (monocytes, T cells and granulocytes) were defined by morphological characteristics based on forward / side scatter subdivision. PBMC subtypes were then further characterized by staining for surface markers and / or intracellular expression of transcription factors.

2.3. Phagocytosis

Phagocytotic function of monocytes and granulocytes was tested using Phagotest™ kit (Glycotope Biotechnology, Heidelberg, Germany) according to manufacturer's instructions. Briefly, one sample of heparinized whole blood was incubated with FITC-labeled *E. coli* at 37 °C, while a negative control sample remained on ice. After adding quenching solution and stopping the phagocytosis reaction, the internalized FITC-labeled bacteria were analyzed by flow cytometry. Percentage of phagocytosing cells and amount of phagocytotic activity was analyzed (percentage of fluorescent cells; MFI).

2.4. Burst reaction

Oxidative burst reaction of leukocytes was determined using Phagoburst™ kit (Glycotope biotechnology) according to manufacturer's instructions. Briefly, heparinized whole blood was incubated with opsonized *E. coli*, PMA (phorbol 12-myristate 13-acetate), or N-fMLP (N-formyl-MetLeuPhe). Production of reactive oxygen species (ROS) by granulocytes and monocytes was indicated by oxidation of the fluorogeneic substrate DHR (dihydrorhodamine 123), and quantified by flow cytometry analysis (percentage of fluorescent cells; MFI).

2.5. S100A12 analysis

S100A12 serum concentrations were determined by a custom made new monoclonal anti-S100A12 ELISA system [20]. All samples were diluted 1:20 and 1:40 to meet the linear range of the assay.

2.6. Statistical analysis

Data were tested for normality using the D'Agostino and Pearson omnibus normality test. Differences in cell phenotypes between untreated patients and controls were compared using Student's *t*-test or Mann Whitney *U* test, as appropriate. Differences in cell phenotypes between untreated and treated patients were analyzed by paired *t*-test or Wilcoxon matched pairs test. Due to the exploratory character of our study, we did not correct for multiple comparisons. *P*-values <0.05 were considered significant. For analysis and figure preparation, we used GraphPad Prism Version 5.0a (GraphPad Software, Inc., San Diego, USA).

3. Results

3.1. Patient data

Patient data are displayed in Table 1. All patients had uveitis complications, whereas none of the patients had undergone ocular surgery. All patients had active IIU at w0. Uveitis activity was well controlled in 6 patients on low-dose steroids (≤0.1 mg/kg) on w12, while in two patients additional immunosuppression with cyclosporine A was initiated due to persisting disease activity.

We compared uveitis patients to data from 28 healthy volunteers. Mean age of healthy controls was 25 years (ranging from 18 to 48 years) with a male/female ratio of 1:3.

3.2. Monocyte phenotyping before corticosteroid treatment

Data respective monocyte phenotyping are displayed in Table 2. We analyzed a broad panel of surface markers necessary for typical monocyte functions, including molecules important for costimulatory (CD80, CD86, MHCII) or regulatory (CD39) function, reception of anti-inflammatory signals (CD121b/IL-1 receptor type II, CD124/IL-4 receptor) and migration (CX3CR1, CCR2).

The percentages of cells positive for the respective markers were not significantly different between patients and healthy controls.

Download English Version:

https://daneshyari.com/en/article/5654959

Download Persian Version:

https://daneshyari.com/article/5654959

Daneshyari.com