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PGE_2/EP4 signaling in peripheral immune cells promotes development of experimental autoimmune encephalomyelitis

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ABSTRACT

Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated inflammatory autoimmune disease model of multiple sclerosis (MS). The inflammatory process is initiated by activation and proliferation of T cells and monocytes and by their subsequent migration into the central nervous system (CNS), where they induce demyelination and neurodegeneration. Prostaglandin E2 (PGE_2) – synthesized by cyclooxygenase 2 (COX-2) – has both pro- and anti-inflammatory potential, which is translated via four different EP receptors. We hypothesized that PGE_2 synthesized in the preclinical phase by peripheral immune cells exerts pro-inflammatory properties in the EAE model. To investigate this, we used a bone marrow transplantation model, which enables PGE_2 synthesis or EP receptor expression to be blocked specifically in peripheral murine immune cells. Our results reveal that deletion of COX-2 or its EP4 receptor in bone marrow-derived cells leads to a significant delay in the onset of EAE. This effect is due to an impaired preclinical inflammatory process indicated by a reduced level of the T cell activating interleukin-6 (IL-6), reduced numbers of T cells and of the T cell secreted interleukin-17 (IL-17) in the blood of mice lacking COX-2 or EP4 in peripheral immune cells. Moreover, mice lacking COX-2 or EP4 in bone marrow-derived cells show a reduced expression of matrix metalloproteinase 9 (MMP9), which results in decreased infiltration of monocytes and T cells into the CNS. In conclusion, our data demonstrate that PGE_2 synthesized by monocytes in the early preclinical phase promotes the development of EAE in an EP4 receptor dependent manner.

1. Introduction

The central nervous system (CNS) is the major target for attack in the human demyelinating disease multiple sclerosis (MS) and in its animal counterpart, experimental autoimmune encephalomyelitis (EAE). Inflammatory processes are the leading cause of CNS demyelination and the subsequent neurological symptoms of MS and EAE [1]. The development of EAE and MS starts in the preclinical phase with activation of T cells in the periphery, followed by activation/proliferation of T cells in the lymph nodes and their migration into the CNS through the blood brain barrier. In the CNS, T cell-secreted cytokines further recruit immune cells (monocytes, B cells) from the blood into the CNS. At this time point, the activation of microglia and astroglia occurs. These inflammatory processes, specifically T cell activation, infiltration of

Abbreviations: BMT, bone marrow transplantation; CFA, complete Freund's adjuvant; COX, cyclooxygenase; cPLA2, cytosolic phospholipase 2; EAE, experimental autoimmune encephalomyelitis; EP, PGE_2 receptor; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; IL, interleukin; LC–MS/MS, liquid chromatography tandem mass spectrometry; MMP9, matrix metalloproteinase 9; MOG, myelin oligodendrocyte protein; MRM, multiple reaction monitoring; MS, multiple sclerosis; PGE_2, prostaglandin E2; PPIA, peptidyl prolyl isomerase; PTX, pertussis toxin.

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inflammatory cells into the CNS and glia activation, promote the formation of lesion sites which characterize the transition to the acute phase and the appearance of clinical symptoms [2].

One major group of mediators that have an important influence on the inflammatory response are prostaglandins (PGs). The biosynthesis of prostaglandins starts with the phospholipase A2 ($cPLA_2$)-induced release of arachidonic acid from the membrane which is subsequently metabolized to PGH_2 by cyclooxygenase-2 (COX-2) or COX-1. PGH2 is converted into PGE_2 by cytosolic or membrane bound PGE_2 synthases. PGE_2 then mediates its effects via activation of four EP receptors (EP1-EP4) and exerts a dual role in inflammatory processes by acting in a pro- or anti-inflammatory manner [3,4].

Previous studies revealed profound alterations in PGE_2 levels and COX-2 expression in CNS-derived samples from MS patients and animals with EAE [5–8], indicating that PGE_2 plays a role in the pathogenesis of MS and EAE. The role of PGE_2 in the development of EAE has already been investigated using either knock-out mice or inhibitors of enzymes of the prostaglandin pathway. The results were contradictory, since the inhibition of COX-2 ameliorates EAE [9], while the complete knock-out of COX-2 has no beneficial effect [10]. Furthermore, an EP4 antagonist applied in the preclinical phase ameliorates symptoms of EAE, while an EP4 agonist only leads to a reduction of EAE symptoms when applied in the acute phase [11]. Our hypothesis is that the origin (peripheral cells/CNS-resident cells) and the time point of synthesis (preclinical phase/acute phase) define whether PGE_2 acts in a pro- or anti-inflammatory manner. We suppose that PGE_2 released by peripheral immune cells is disease-promotion.

To investigate the role of peripheral and CNS-resident cells, the bone marrow transplantation model (BMT) was used. Bone marrow-derived immune cells (peripheral cells), such as lymphocytes and monocytes/macrophages, play a major role in mediating inflammatory processes in multiple sclerosis. The transplantation of bone marrow cells between animals with different genetic backgrounds (i.e. knock-out mice) allows the role of specific proteins in myeloid/lymphoid cell function to be investigated. The BMT model was used to specifically deplete COX-2 or EP receptors in peripheral blood cells (lymphocytes, monocytes), while leaving cells of the CNS (microglia, astroglia, oligodendrocytes) and all other tissue cells with normal COX-2 or EP expression or vice versa.

Our data suggest that PGE_2, synthesized by peripheral monocytes, promotes the development of EAE in an EP4 dependent manner. This was indicated by a delay in the onset of EAE in mice lacking COX-2 or EP4 in bone marrow-derived cells. This effect was associated with a reduced level of the T cell activating interleukin-6 (IL-6) and a reduced number of T cells and T cell secreted interleukin-17 (IL-17) in the blood of mice lacking COX-2 or EP4 in peripheral immune cells, respectively. Furthermore, EAE mice lacking COX-2 or EP4 in peripheral blood cells showed a reduced matrix metalloproteinase 9 (MMP9) level which was accompanied by a reduced infiltration of monocytes and T cells into the CNS and the absence of lesion sites in the CNS.

2. Materials and methods

2.1. Cells and reagents

PGE_2 was purchased from Cayman Chemical (USA). The EAE (EK-0115) and the control (CK-0115) kit were purchased from Hooke Laboratories (Lawrence, USA).

2.2. Animal models

In all experiments, the ethics guidelines for investigations in conscious animals were obeyed and the experiments were approved by the local Ethics Committee for Animal Research.

2.2.1. Induction and evaluation of BMT

Ten-week-old female C57BL/6 weighing 18–20 g were obtained from Harlan Laboratories (Horst, Netherlands). The C57BL6-Tg(UBC-GFP)30Scha/J (GFP, green fluorescence protein) mice were obtained from Charles River (Köln, Germany). The COX-2KO, EP1KO, EP2KO, EP3KO and EP4KO mice were a gift from Prof. Nüsing. For the generation of chimeric mice the bone marrow stem cells were washed out of the tibia and femur of the donor mice (COX-2KO, EP1KO, EP2KO, EP3KO, EP4KO, GFP). Host mice (WT, COX-2KO) were irradiated (9.5 Grey) and, subsequently, 3×10^6 bone marrow cells isolated from the donor mice were injected into the tail vein. Using COX-2KO mice as donor and WT mice as host chimeric mice denoted as COX-2KO → WT were generated. Therefore, COX-2KO → WT mice lacking COX-2 expression in the peripheral cells, while WT → COX-2KO mice lacking COX-2 expression in all tissue cells including CNS-resident cells. Three weeks after the transplantation, the success of the transplantation was verified by PCR (polymerase chain reaction) in the case of knock-out mice as donors and by fluorescence-activated cell sorting (FACS) in the case of GFP mice as donors.

2.2.2. Induction and evaluation of EAE

Ten-week-old female C57BL/6 mice or BMT mice (4 weeks after BMT) were used for the induction of EAE. The EAE induction was conducted as recommended by Hooke Laboratories (Lawrence, USA) and was described previously [12]. Briefly, mice anesthetized using isoflurane (2% in carbogen gas) received a subcutaneous injection into the upper and lower back of the encephalitogenic MOG_{35-55} (myelin oligodendrocytes protein) peptide (200 μg), emulsified in complete Freund's adjuvant (CFA) containing 400 μg *Mycobacterium tuberculosis*. Two hours thereafter, and again 24 h later, the mice received an intraperitoneal injection of pertussis toxin (PTX). Since CFA alone also exerts inflammatory effects [13], two control groups were used. In one group (untreated group) mice received neither CFA/PTX nor MOG and in the other group (CFA control) mice were treated once with CFA-containing *M. tuberculosis* and twice with PTX. One week after the injection, the mice were examined daily for developing clinical symptoms. All BMT-EAE mice developed clinical symptoms. For each experiment control WT → WT-EAE mice run in parallel since the day of disease onset varied in the different experiments. Clinical symptoms were classified by clinical scores as follows: sc0) no signs, sc0.5) limp tail, sc1) limp tail and weakness of hind legs, sc2) limp tail and paresis of hind legs, sc3) limp tail and paralysis of hind legs.

2.2.3. Combination of BMT and EAE model

For a proof of principle for the combination of BMT and EAE model, bone marrow cells from GFP mice were isolated and subsequently injected into irradiated wild type mice. These mice were referred to GFP → WT mice. After three weeks, the blood cells were analyzed using fluorescence-activated cell sorting (FACS). As expected, about 90% of the blood cells from GFP → WT mice expressed GFP indicating that the transplantation was successful. After four weeks, the EAE model was induced. Immunohistochemistry data from GFP → WT-EAE mice (with sc3) show that blood-derived-GFP-labeled immune cells had infiltrated the lumbar spinal cord.

2.3. Plasma from patients

Plasma from patients was collected in the Department of Neurology (Goethe-University, Frankfurt/Main). All patients gave written informed consent prior to participation and the protocol was approved by the local ethics committee. All samples were stored at −80 °C. Plasma samples were collected from 10 MS patients and 10 healthy controls. The patients were diagnosed with