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Inhibition of effector antigen-specific T cells by intradermal administration of heme oxygenase-1 inducers

Thomas Simon [a, c, f], Julien Pogu [a], Séverine Rémy [a], Frédéric Brau [b], Sylvie Pogu [c], Maud Maquigneau [a], Jean-François Fonteneau [d], Nicolas Poirier [a, g], Bernard Vanhove [a], Gilles Blancho [a], Eliane Piaggio [e], Ignacio Anegon [a, 1], Philippe Blancou [a, b, c, *, 1]

[a] Université de Nantes, Inserm, UMR1064, Center for Research in Transplantation and Immunology, 44093, Nantes, France
[b] Université Côte d'Azur, Inserm, CNRS, IPMC, 06560, Valbonne, France
[c] INRA USC1383, IECM; LUNAM Université, Oniris, Nantes, EA4644, France
[d] Université de Nantes, Inserm, UMR892, CNRS UMR6299, 44007, Nantes, France
[e] Institut Curie, Inserm, UMR932, 75005, Paris, France
[f] Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[g] OSE Immunotherapeutics, 44200 Nantes, France

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ABSTRACT

Developing protocols aimed at inhibiting effector T cells would be key for the treatment of T cell-dependent autoimmune diseases including type 1 autoimmune diabetes (T1D) and multiple sclerosis (MS). While heme oxygenase-1 (HO-1) inducers are clinically approved drugs for non-immune-related diseases, they do have immunosuppressive properties when administered systemically in rodents. Here we show that HO-1 inducers inhibit antigen-specific effector T cells when injected intradermally together with the T cell cognate antigens in mice. This phenomenon was observed in both a $CD8^+$ T cell-mediated model of T1D and in a $CD4^+$ T cell-dependent MS model. Intradermal injection of HO-1 inducers induced the recruitment of HO-1^+ monocyte-derived dendritic cell (MoDCs) exclusively to the lymph nodes (LN) draining the site of intradermal injection. After encountering HO-1^+MoDCs, effector T-cells exhibited a lower velocity and a reduced ability to migrate towards chemokine gradients resulting in impaired accumulation to the inflamed organ. Intradermal co-injection of a clinically approved HO-1 inducer and a specific antigen to non-human primates also induced HO-1^+ MoDCs to accumulate in dermal draining LN and to suppress delayed-type hypersensitivity. Therefore, in both mice and non-human primates, HO-1 inducers delivered locally inhibited effector T-cells in an antigen-specific manner, paving the way for repositioning these drugs for the treatment of immune-mediated diseases.

1. Introduction

Organ-specific autoimmune disease such as type 1 diabetes (T1D) and multiple sclerosis (MS) are induced by the recruitment of activated auto-reactive $CD4^+$ and $CD8^+$ T cells to target organs. When autoimmunity is diagnosed, pathogenic T-cells have already homed to the target organ, therefore leaving a narrow therapeutic window for immuno-intervention before irreversible damages prevail [1]. While systemic administration of antibodies directed to CD3 [2], CD20 [3], CTLA-4 [4] (for T1D), and α4 integrin [5] (for MS) has resulted in some clinical improvement, these antibody-based treatment have resulted in general immuno-suppression and increased risks of opportunistic infections [6]. As an alternative approach, several investigators have tried to develop therapeutic protocols aimed at inhibiting antigen-specific auto-reactive effector T cells. Indeed, the oral administration of autoantigens to pre-diabetic non-obese-diabetic (NOD) mice prevented the development of T1D [7] or inhibited MS-like clinical symptoms in the experimental autoimmune encephalomyelitis (EAE) model [8]. Unfortunately, clinical trials based on these approaches have been disappointing (for review see Ref. [9]). Thus, new therapeutic strategies aimed to selectively targeting antigen-specific T cells are urgently needed.

Heme oxygenase (HO) catalyzes the degradation of free heme to

* Corresponding author. Université de Nice Sophia Antipolis, CNRS, INSERM, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, 06560, France.
E-mail address: blancou@ipmc.cnrs.fr (P. Blancou).
[1] Co-senior authorship.

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carbon monoxide (CO), iron, and biliverdin. Among the three known HO isoforms, HO-1 is the only one induced by inflammatory and chemical stimuli. Several HO-1-inducers including cobalt protoporphyrin (CoPP) and the clinically approved drug Normosang® have been identified and demonstrated to exert anti-inflammatory properties when administered systemically to rodents [10,11]. Likewise, the systemic induction of HO-1 prevented both T1D in NOD mice [12,13] and EAE [14]. While the mechanism by which HO-1 exerted this protective effect remains to be elucidated, it was definitively not antigen-specific since antigen is dispensable to mediate the protection although HO-1$^+$ antigen-presenting cells (APCs) are purported to be detrimental to this effect [14–17]. Furthermore, systemic administration of HO-1 inducers for prolonged periods would have deleterious effects [18].

In this study, we hypothesized that HO-1 inducers selectively inhibits antigen-specific T cells when injected intradermally, and thus presented locally, together with a defined antigen. Our results in two mouse models of autoimmunity and a primate model of Delay Type Hypersentitivity (DTH) showed that this was indeed the case. Following HO-1 inducers and antigen intradermal administration, we identified an antigen-bearing HO-1$^+$ monocyte-derived dendritic cell (MoDC) population that appears in the draining lymph node of the intradermal injection site, and that tolerizes pathogenic T-cells by inhibiting their migration to target organs.

2. Materials and methods

2.1. Cells

2.1.1. Autoreactive CTL generation

Autoreactive CTLs were generated as described previously [19]. Briefly, CD8$^+$ cells were isolated by magnetic selection (Miltenyi Biotech) from OVA-specific class I-restricted T cells (OT-I) mice [20] spleen and lymph node single-cell suspensions. 1×10^6 purified OT-I CD8$^+$ T cells were stimulated with 5×10^6 mitomycin-treated and ovalbumin (257–264) peptide–loaded syngeneic spleen cells in 2 ml complete DMEM high glucose with stable glutamine (PAA) supplemented with 10% FCS (Eurobio) containing, 5 ng/ml IL-2 (Roche Applied Science), and 20 ng/ml IL-12 (R&D Systems). On day 3, the cultures were split into four aliquots and fed with fresh medium containing IL-2. On day 6, cells were collected and washed with culture medium at three times.

2.1.2. Isolation of murine APCs and co-culture with autoreactive CTLs

For MHC-II$^+$ and F4/80$^+$ cells isolation, skin-draining LNs of the injection site were removed 24 h after intradermal injection in the back or in the ear. Single-cell suspensions were prepared by enzymatic lymph node disaggregation with collagenase D (Sigma-Aldricht). Cells were stained with anti-MHC-II-FITC (clone AF6-120.1, BD pharmingen) or anti-F4/80-PE (clone BM8, eBioscience) monoclonal antibodies as primary labeling reagent and respectively with anti-FITC or anti-PE microbeads (Miltenyi) as secondary reagent. After magnetic separation, we checked that purity was >95% for CD11b$^+$Ly6C^{high}F4/80$^+$ cells in CoPP-OVA immunized mice. These cells were co-cultured with autoreactive CTLs (respectively 1:1 for MHC-II$^+$ and 1:5 for F4/80$^+$ APC to CTL ratio) overnight for velocity measurement and transwell migration assay.

2.1.3. Human THP-1 monocytic cell line and co-culture with CD8$^+$ T cells clones

THP-1 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Eurobio), 1% glutamine (Gibco), 50 IU/mL penicillin 50 IU/mL streptomycin (Gibco) and 200 nM PMA (Sigma-Aldrich) under standard conditions as previously described [21].

For co-culture experiments with CD8$^+$ T cells clones, THP-1 were treated with CoPP (12.5 μM or 25 μM) overnight, pulsed 30 min at 37 °C with 5 μM of MUC1 (950–958) in culture medium, thoroughly washed and cocultured with HLA-A*0201/MUC1(950–958)-specific T-cell clone (N5.14) [22] at ratio 1:1 for velocity measurement (see below).

2.2. Animals

2.2.1. Non-human primates

Baboons (*Papio anubis*, from the CNRS Primatology Center, Rousset, France) were negative for all quarantine tests. All experiments were performed under general anaesthesia with Zoletil (Virbac, Carron, France). Three Baboons were injected intradermally in the inguinal fold with respectively 6.25 mg (500 μL), 12.5 mg (500 μL) or 25 mg (1 mL) of clinical hemin (Normosang®). A non-treated baboon has served as control. Inguinal lymph nodes were surgically removed 24 h after intradermal injection. Single-cell suspensions for flow cytometry analysis were prepared by enzymatic lymph node disaggregation with Collagenase D (Sigma-Aldricht).

2.2.2. Mice

The RIP-OVA^{high} transgenic mice [23] express OVA in pancreatic islets and the OT-I CD45.1$^+$ transgenic mice express a TCR-specific for the H2Kb restricted (SIINFEKL) epitope of OVA and the CD45.1 congenic marker. 2D2 mice express a TCR-specific for the H-2b restricted (MEVGWYRSPFSRVVHLYRNGK) epitope of Myelin Oligodendrocyte Glycoprotein (MOG) [30]. Mice were obtained through Jackson Laboratory (RIP-OVA^{high}, 2D2), Charles River (OT-I CD45.1$^+$, NOR and NOD) or Janvier (C57/BL6). All mice were used between 6 and 12 weeks of age.

2.3. Intradermal immunization of mice

Eight to ten week-old RIP-OVA^{high} mice received two intradermal injections in the back with 70 μg of CoPP (Livchem) or/and 140 μg of MnPP (Livchem) prepared as described [16] and 20 μg of endofree ovalbumin (Hyglos) in 10 μl. Forty micrograms of Alexa Fluor® 488 ovalbumin (Molecular Probes) has been used for phagocytosis assay.

2.4. Diabetes induction in RIP-OVA^{high}

For diabetes induction, RIP-OVA^{high} mice were injected i.v. with 0.5×10^5 autoreactive cytotoxic OT-I CD8$^+$ T cells (purity >95%). In some experiments, OT-I CD8$^+$ T cells were previously co-cultured or not with MHC-II$^+$ or F4/80$^+$ cells during 16 h. Glycemia was monitored every days starting from day five. Mice were considered diabetic when blood glycemia was superior to 180 mg/dL during two consecutive days.

2.5. EAE induction

As describe in Ref. [14], EAE was induced in C57BL/6 by subcutaneous injection of emulsified complet freund adjuvant (CFA) (Sigma Aldrich) complemented with 400 μg of mycobacterium tuberculosis (BD Biosciences) and 200 μg of MOG$_{35-55}$ ClassII peptide (GeneCust). Two-hundred nanogram of pertussis toxin (VWR International) were intravenously injected at the time of immunization and two days later. Clinical signs of EAE were evaluated daily and scored as follows: 0, normal; 1, limp tail; 2, partial paralysis of the hind limbs; 3, complete paralysis of the hind limbs. For prophylactic treatment, mice were treated or not at the time of EAE induction