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Research paper

Long-term sustainable dendritic cell-specific depletion murine model for periodontitis research

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ABSTRACT

Dendritic cells (DCs) are specialized antigen-presenting cells that play a pivotal role in the pathogenesis of periodontitis. The use of animal models to study the role of DCs in periodontitis has been limited by lack of a method for sustained depletion of DCs. Hence, the objectives of this study were to validate the zDC-DTR knockin mouse model of conventional DCs (cDCs) depletion, as well as to investigate whether this depletion could be sustained long enough to induce alveolar bone loss in this model. zDC-DTR mice were treated with different dose regimens of diphtheria toxin (DT) to determine survival rate. A loading DT dose of 20 ng/bw, followed and maintained with doses of 10 ng/bm every 3 days for up to 4 weeks demonstrated 80% survival. Animals were weighed weekly and peripheral blood was obtained to confirm normal neutrophil counts. Five animals per group were euthanized at baseline, 24 h, 1 and 4 weeks. Bone marrow (BM), spleen (SP) and gingival tissue (GT) were harvested, and cells were isolated, separated and stained for Pre-DCs precursors ($CD45R^-MHCII^+CD11c^+Flt3^+CD172a^+$) in BM, cDCs ($CD11c^+MHCII^+CD209^+$) in spleen, and DCs in GT ($CD45R^+MHCII^+CD11c^+$ DC-SIGN/$CD209^+$). Pre-DCs in BM were significantly depleted at 24 h and depletion maintained for up to 4 weeks, as compared to blank (PBS) controls. Circulating cDCs in spleen demonstrated a non-significant trend to deplete in 1 week with high variability among mice. GT also showed a similar non-significant trend to deplete in 24 h. The zDC-DTR model seems to be viable for evaluating the role of DCs immune homeostasis disruption and alveolar bone loss pathogenesis in response to long-term oral infection.

1. Introduction

Dendritic cells (DCs) are the most important specialized antigen-presenting cells and essential mediators of the immune response. DCs activate naïve T cells, linking innate and adaptive immune responses, as well as exerting a critical influence on the intensity and quality of the inflammation (Geissmann et al., 2010). DCs are differentiated by their origin, function and location. Resident dendritic cells (i.e. Langerhans cells) have long lifespans and play an active role in immune surveillance, promoting host tolerance or immunity (El-Awady et al., 2015). However, nearly 50% of the DCs are migratory subsets rather than typical resident DCs. DCs can also display a high level of plasticity, as exemplified by the ability of some DCs subsets to become osteoclast precursors (Miyamoto et al., 2001; Cutler and Teng, 2007). Mucosal DCs present in the gingival epithelium and connective tissue have been shown to be highly responsive to the accumulation of bacterial plaque, migrating into the colonized sites during gingivitis and periodontitis (Jotwani et al., 2001; Jotwani and Cutler, 2003; Jotwani et al., 2004; Upadhyay et al., 2013) and forming immune conjugates with CD4+ T cells in connective tissues (Jotwani et al., 2001). However, the precise role of DCs in mediating immunity, tolerance or pathogenesis in periodontitis is still unclear (Cutler and Jotwani, 2006).

The connection between inflammation and bone destruction has been well established in numerous clinical and animal studies, and reviewed elsewhere (Hardy and Cooper, 2009). In this scenario, DCs emerge as pivotal inflammatory cells that are highly plastic depending on microenvironment in which they arrive; for example, DCs can be matured in response to bacterial infection, can reprogram their chemokine receptors, disrupting homing mechanisms, but facilitating migration towards inflammatory vascular sites, where DCs can resist to apoptosis (Zeituni et al., 2010; Miles et al., 2013; Miles et al., 2014). Moreover, a clinical study showed that myeloid DCs could be expanded in numbers in the peripheral blood of subjects with chronic periodontitis when compared to healthy subjects (Carrion et al., 2012).

DCs manipulation in mice emerges as an effective model to study their role in periodontal pathogenesis. However, functional DCs studies have faced an important obstacle: both DCs and monocytes/macrophages arise from a common myeloid precursor cell in the bone marrow

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- the macrophage and DC progenitor cell (MDP) (Fogg et al., 2006), which gives rise to the common DCs progenitors (CDP). DCs further differentiate into different subsets, the two major being conventional DCs (cDCs) and plasmacytoid DCs (pDCs) (Naik et al., 2007; Onai et al., 2007). As a result of this shared origin, monocytes originating from MDP will present a convergence on the phenotype with DCs, including the expression of integrin alpha X (CD11c) and major histocompatibility complex class II (MHCII) (Hashimoto et al., 2011). This convergence makes it difficult to distinguish the precise function of DCs in knockin/knockout models, and whether DCs have a specific function different from other CD11c-expressing cells (Hume, 2008). In an attempt to study the function of DCs in vivo, mouse models for specific depletion of DCs have been developed over the years (Durai and Murphy, 2016). The diphtheria toxin (DT) based models, in which the DT receptor (DTR) is spliced in the mouse genome under the control of certain transcription factors, allows a conditional short-term depletion of DCs in vivo (Saito et al., 2001; Jung et al., 2002). One of the most extensively used DTR models is the CD11c-DTR (Jung et al., 2002; Probst et al., 2005), which has been used to study the function of cDCs in different diseases, but this model has fell short in promoting the specific depletion of cDCs (Bennett and Clausen, 2007; van Blijswijk et al., 2013; Karmaus and Chi, 2014). Recently, the zDC-DTR model, in which the DTR is placed under the control of the zinc finger transcription factor zDC (which is specifically expressed by cDCs but not monocytes or other immune populations), has shown promising results in short-term specific depletion of cDCs (Meredith et al., 2012). The expression of this zDC zinc finger transcription factor identifies the earliest committed precursors of cDCs (Pre-DCs), and it was found to be elevated in CD8+ and CD4+ subsets of cDCs, but not in pDCs, monocytes, granulocytes, and T and B cells. By inserting a human DTR cDNA into the 3'UTR of the zDC gene, the treatment with DT results in specific depletion of cDCs in the knock-in mouse (Meredith et al., 2012; Satpathy et al., 2012). DT models, however, have some disadvantages including lethality, monocytosis, neutropenia/neutrophilia or the depletion of additional cell populations. A way to compensate for these limitations is the use of radiation chimeras (Tittel et al., 2012; van Blijswijk et al., 2013).

Most DC knockin models have been shown to have immediate short-term effects on depletion, however many experimental conditions like murine alveolar bone loss require long-term exposure to oral bacteria (2–4 weeks in average) (Graves et al., 2008; Graves et al., 2012). zDC-DTR has been reported to maintain depletion of cDCs with low doses of DT every 3 days in short-term experiments (Meredith et al., 2012; Behler et al., 2015; Loschko et al., 2016; Dommaschk et al., 2017). However, a recent study using low DT dose in chimeras failed to maintain long-term depletion in 18 weeks (Rombouts et al., 2017).

Currently, to the best of our knowledge, there are no reports experimentally addressing the specific role of DCs depletion in the pathogenesis of periodontitis. In this scenario, the objectives of this study were to validate the long-term zDC-DTR knockin mouse model of conditional cDC depletion as well as to investigate if this model could be of use for murine alveolar bone loss models. The hypothesis of this study was that the depletion of pre-DCs in BM and, consequently, the depletion of cDCs in spleen could be maintained for up to 4 weeks, in accordance with most oral infection models (Graves et al., 2008).

2. Methods

The Augusta University Institutional Animal Care and Use Committee approved the protocol for this study (approval number 2013-0586, 10/11/2013).

2.1. Mice

For this study, males and females C57BL/6 (B6) and C57BL/6-Zbtb46$^{tm1(DTR)Mnz/J}$ (zDC-DTR) knockin mice, 17–20-week age, were purchased from The Jackson Laboratory (Sacramento, CA) and a colony was expanded for experimentation. Mice were housed in the Division of Laboratory Animal Services at Augusta University, in a group of up to 5 animals per plastic cage, labeled with cage cards. The cages were housed in purpose-designed air-conditioned rooms with temperature (18–22 °C) and relative humidity (30–70%) monitored daily. A 12/12 h light/dark cycle was used. The animals had ad libitum access to water and a standard sterile laboratory diet.

2.2. Dosage versus survival rates

DT was purchased from Sigma-Aldrich (#D0564), suspended in aliquots and stored in −20 °C. To establish which dosage could be considered as ideal (highest possible dose in which the animals would survive for up to 4 weeks), 3 groups of 5 zDC-DTR mice each (randomly distributed in males and females) were followed with different dosages to verify survival rates. For all animals, a loading dose of 20 nanograms per gram of body weight (ng/bw) of DT was administrated intraperitoneal (i.p.), followed by maintenances doses of 4 (DT20/4), 10 (DT20/10) or 20 (DT20/20) ng/bw every 3 days. B6 animals were also treated with DT20/10 protocol.

2.3. Study design and samples

For cDCs depletion experiment, B6 and zDC-DTR animals were injected i.p. with an initial loading dose of 20 ng/bw, followed by maintenance doses of 10 ng/bw every 3 days, for up to 4 weeks (B6 DT-treated and zDC-DTR DT-treated groups). The control group consisted of zDC-DTR mice treated with 100ul of saline solution every 3 days for up to 4 weeks (zDC-DTR blank control). Animals were weighed weekly. Neutrophil counts were determined by morphology after Wright's stain (Eng Scientific, #2500) from extended peripheral blood obtained via tail nick from 5 animals at baseline, 24 h, 1 and 4 weeks, by a trained and masked examiner (AFS). Percentages of neutrophils were calculated based on the counting of 200 cells.

Five zDC-DTR DT-treated and 5 zDC-DTR blank control animals were euthanized per time point (baseline, 24 h after a loading DT dose and at 1 and 4 weeks of treatment, CO_2 inhalation followed by decapitation). Bone marrow (BM) and spleen (SP) were harvested and cells were isolated following a protocol previously described (Madaan et al., 2014). At baseline, 24 h, and 1 week, gingival tissue (GT) was also harvested and cells were isolated following a modified protocol from a previously published method (Mizraji et al., 2013). The modification consisted of processing the samples using a TissueLyser (QIAGEN, Hilden, Germany) for 15 s, 15 Hz, before incubation with DNAse/collagenase cocktail. Time of incubation was also changed to 30 min, followed by 15 min with EDTA.

2.4. Lost animals

All lost animals were replaced during the experiment. For one of the animals lost during the course of the study, a necropsy was performed to investigate death causes. The necropsy was performed by a veterinarian pathologist (Veterinary Diagnostic Laboratories, The University of Georgia), and consisted of a gross pathology and histopathology of brain, lung, trachea, larynx, thyroid, heart, kidney, tongue, liver, spleen, pancreas, small and large intestines, and preputial gland.

2.5. Antibodies and other reagents

Anti-MHCII, VioGreen (#130-106-363) was acquired from Miltenyi Biotec. Anti-CD172a, FITC, was acquired from Biolegend, (#144006). The following antibodies were acquired from eBioscience: anti-CD45.R, eFluor 450 (#48-0452-80); anti-Flt3, PE-Cy5 (#15-1351-81); anti-CD11c, APC (#17-0114-82); anti-CD14, FITC (#11-0141-82); and anti-CD209, PE (#12-2091-82). Fixation Buffer (#00-8222-49) and Flow Cytometry Staining Buffer Solution (#00-4222-26) were also