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B cells are required for sunlight protection of mice from a CNS-targeted autoimmune attack

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ABSTRACT

The ultraviolet (UV) radiation contained in sunlight is a powerful immune suppressant. While exposure to UV is associated with protection from the development of autoimmune diseases, particularly multiple sclerosis, the precise mechanism by which UV achieves this protection is not currently well understood. Regulatory B cells play an important role in preventing autoimmunity and activation of B cells is a major way in which UV suppresses adaptive immune responses. Whether UV-protection from autoimmunity is mediated by the activation of regulatory B cells has never been considered before. When C57BL/6 mice were exposed to low, physiologically relevant doses of UV, a unique population of B cells was activated in the skin draining lymph nodes. As determined by flow cytometry, $CD1d^{low}CD5^{-}MHC\text{-}II^{hi}B220^{hi}$ UV-activated B cells expressed significantly higher levels of CD19, CD21/35, CD25, CD210 and CD268 as well as the co-stimulatory molecules CD80, CD86, CD274 and CD275. Experimental autoimmune encephalomyelitis (EAE) in mice immunized with MOG/CFA was reduced by exposure to UV. UV significantly inhibited demyelination and infiltration of inflammatory cells into the spinal cord. Consequently, UV-exposed groups showed elevated IL-10 levels in secondary lymphoid organs, delayed EAE onset, reduced peak EAE score and significantly suppressed overall disease incidence and burden. Importantly, protection from EAE could be adoptively transferred using B cells isolated from UV-exposed, but not unirradiated hosts. Indeed, UV-protection from EAE was dependent on UV activation of lymph node B cells because UV could not protect mice from EAE who were pharmacologically depleted of B cells using antibodies. Thus, UV maintenance of a pool of unique regulatory B cells in peripheral lymph nodes appears to be essential to prevent an autoimmune attack on the central nervous system.

1. Introduction

Insufficient exposure to the ultraviolet (UV) radiation contained in sunlight is a major aetiological risk factor in the development of a range of autoimmune diseases including type 1 diabetes [1], Crohn's disease [2], colitis [3] and possibly rheumatoid arthritis [4]. Decreased incidence of central nervous system (CNS)-autoimmune diseases such as multiple sclerosis (MS) show the most striking correlation with increased exposure to UV [5–8]. MS patients often present with low Vitamin D serum levels [9] and exposing the skin to UV is the most efficient way to make the active form of Vitamin D, so these two events may be linked. However, UV exposure and vitamin D status are independent risk factors for MS [10] indicating that something additional to the active form of Vitamin D that is triggered by UV contributes to the protection afforded by maintaining adequate levels of sunlight exposure [11].

Mice exposed to UV are protected from the CNS-targeted autoimmune disease experimental autoimmune encephalomyelitis (EAE) [12]. This protection is independent of UV-induced Vitamin D [13] and involves, in part, regulatory T cell-mediated immune suppression [14]. The UV-induced immune suppressive antimicrobial peptide, β-defensin-14 (mBD14) may also be involved [15]. Exposure to solar UV suppresses the induction, effector and memory phases of $CD8^{+}$ cytotoxic T lymphocyte (CTL) responses [16], as well as $CD4^{+}$ T helper cell (Th) type 1 (Th_1) [17], Th_2 [18],

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Th_{17} [19] and even T follicular helper (T_{FH}) [20] responses. UV-immunosuppression may therefore explain how sunlight exposure is associated with protection from some T cell-driven autoimmune diseases like MS [21].

A large number of cellular and molecular events occur after exposure to UV. These local and systemic events converge on the activation of regulatory cells in secondary lymphoid tissues that suppress inflammation and regulate adaptive immune responses. In addition to UV-induced T_{Regs} [22], Natural Killer (NK)-T cells [23] and mast cells [20,24–26], a major way UV causes immune suppression is via the activation of an MHC-$II^{hi}B220^{hi}$ regulatory B cell [27–29]. Mice deficient in B cells develop more severe EAE [30] and it is now widely appreciated that regulatory B cells (B_{Regs}) play a pivotal role in the prevention [31–34] and treatment [35,36] of EAE. In humans, there is also a strong link between B cell production of IL-10 and protection from CNS-autoimmunity [37]. Indeed, MS patients in relapse had 5–6 fold lower numbers of circulating IL-10-producing B cells compared to those in remission [38]. Importantly, when these MS patients remitted, the numbers of "B_{Regs}" had returned to normal. Thus, B cells are also involved in protecting humans from MS.

Here we show that an EAE-protecting UV regime activates a unique subset of B cells in skin draining lymph nodes and that this activation correlates with successful protection from EAE. Mice that were injected with a B cell-depleting antibody were no longer protected from EAE by UV, while adoptive transfer of B cells isolated from UV-exposed, but not unirradiated hosts conferred protection from EAE. Together these results confirm that UV-activation of immune regulatory B cells is a major cellular event in protection from a CNS-targeted autoimmune attack.

2. Materials and methods

2.1. Mice and exposure to UV radiation

8-10 week old female C57BL/6 mice (Animal Resource Centre, Perth, WA, Australia) were used with approval from the University of Sydney animal ethics committee (K14/5-2010/3/5336 and K14/7-2013/3/6020). Back hair was shaved off 24 h prior to the first UV exposure. For B cell phenotypic analysis, mice were exposed to 3 daily doses of solar-simulated UV (1.46 J/cm^2 per day) using a 1000 W xenon arc lamp solar simulator (Oriel, Stratford, CT, USA) that delivers a spectrum that is almost identical to the UV region within sunlight [39]. 72 h following the final exposure to UV, which corresponds to the time that regulatory B cells appear in UV-exposed mice [27], the back-skin draining (inguinal) lymph nodes were removed and single cells prepared for phenotypic analysis by flow cytometry. To determine whether exposure to UV can protect from EAE, mice were exposed to 3.64 J/cm^2 of solar simulated UV daily for 7 consecutive days at which point EAE was induced (day 0). UV was continued every 2nd day until the end of the experiment. Control mice were shaved and restrained but did not receive UV.

2.2. Modelling CNS-autoimmunity using experimental autoimmune encephalomyelitis (EAE)

EAE was induced in 8–10 week old C57BL/6 female mice using 1 μg/μL MOG peptide (MOG_{35-55}, MEVGWYRSPFSRVVHLYRNGK; ProSpec, Rehovot, Israel) emulsified at a 1:1 ratio in complete Freund's adjuvant (CFA; Sigma-Aldrich, St Louis, MO). Each mouse was immunized subcutaneously into both sides of the abdomen (2 × 40 μL) and tail base (2 × 10 μL). In some experiments, additional control groups included mice immunized with PBS/CFA emulsion. Sixty minutes as well as 48 h after the immunization, mice received an intravenous tail vein injection of 200 ng Pertussis toxin (Sigma-Aldrich) in 200 μL sterile PBS. Body weight was measured daily following immunization and EAE clinical disease was scored as follows: 0; no disease, 1; loss of tail tonus, 1.5; impaired righting reflex, 2; hind limb weakness, 2.5; one hind leg paralyzed, 3; both hind legs paralyzed with residual mobility in both legs, 3.5; both hind legs completely paralyzed, 4; front limb paralysis/weakness. A body weight loss of greater than 15% or a clinical score of 4 required euthanasia for ethical reasons.

2.3. Isolation and analysis of CNS tissue

Mice were perfused with 4 °C PBS before collecting the brain and spinal cord. Isolated whole spinal cord (~90–130 mg) was homogenized in Trizol using 1 mm zirconia beads (BioSpec Products Inc., Bartlesville, USA) before centrifugation at 12,000*g*. mRNA was purified using an RNeasy MiniKit (Qiagen, Venlo, Netherlands) before being synthesized into cDNA. Primers to **CXCL12** (F: CCACCATGGAGAAGGCTGGGGCTC, R: AGTGATGGCATGGACTGTGGTCAT), **CXCR4** (F: GTGGCTGACCTCCTCTTTGT, R: TTTCAGCCAGCAGTTTCCTT) and **CXCR7** (F: CTGGTATGTTGGCTGCCGTA, R: GGCCTTCATCAGCTCGTACC) were used to assess mRNA levels by real time quantitative RT-PCR which consisted of 45 cycles, each containing 20 s of denaturing and annealing at 95 °C, followed by extension at 64 °C for 40 s. Genes of interest were normalized to GAPDH (F: CCACCATGGAGAAGGCTGGGGCTC, R: AGTGATGGCATGGACTGTGGTCAT) using the $2^{-\Delta\Delta(C)_T}$ method as we have previously described [26].

For experiments involving flow cytometry on single cells, freshly isolated and homogenized CNS tissue was incubated with DNase I (280U per sample, Sigma-Aldrich) and collagenase E (5 mg per sample, Sigma-Aldrich) for 1 h at 37 °C during which time the homogenate was re-suspended by pipetting up and down every 15 min. The filtered homogenate was centrifuged at 478*g* for 10 min before being resuspended in 7 mL of 30% Percoll (Sigma-Aldrich) containing 10% fetal bovine serum in complete RPMI. This 30% Percoll suspension was overlaid onto 3 mL of 80% Percoll and centrifuged at 1665*g* for 25 min at 22 °C with no brake to isolate single cells at the interface. Washed single cells were incubated with Fc block (CD16/CD32, clone 93 from eBioscience; San Diego, CA) for one hour at 4 °C, before being stained with UV-Live/Dead (Invitrogen, Carlsbad, CA) followed by incubation with the following panel of antibodies (clones): B220 (RA3 6B2), CD45 (30-F11), CD4 (GK1.5), MHC II (M5/114.15.2) (all from Biolegend; San Diego, CA), CD11b (M1/70), CD19 (1D3), CD44 (1M7), CD8 (53–6.7), CXCR4 (2B11) (all from eBioscience).

2.4. Phenotypic analysis of cells by flow cytometry

Single cell suspensions of spleen as well as skin-draining (inguinal) and CNS-draining (lumbar) lymph nodes were prepared as described [27] and stained with anti-mouse CD1d (1B1), CD5 (53–7.3), CD19 (1D3), CD25 (PC61) (all from Becton Dickinson; Franklin Lakes, USA), B220 (RA3-6B2), MHC II (M5/114.15.2), CD21/35 (7E9), CD79b (HM79-12), CD86 (PO3), CD210 (1B1.3a), CD268 (7H22-E16), CD274 (10F.9G2), CD275 (HK5.3), CD276 (RTAA15) (all from Biolegend), and CD80 (16-10A1) (eBioscience). Fluorescence minus one controls were used to set all electronic gates. Acquisition was performed on either a FACSCanto or LSR-Fortessa flow cytometer (Becton Dickinson). All flow cytometry data was analyzed using FlowJo software v8.8.7 (Treestar Inc., Ashland, OR, USA).