Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/cei.12817

Plasmacytoid dendritic cells and type 1 interferon promote peripheral expansion of forkhead box protein 3^+ regulatory T cells specific for the ubiquitous RNA-binding nuclear antigen La/Sjögren's syndrome (SS)-B

Z.-J. Pan,*[1] C. G. Horton,*†‡[1] C. Lawrence* and A. D. Farris*†
*Arthritis and Clinical Immunology Program, Oklahoma Medical Research Foundation, †Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, and ‡Department of Biological Sciences, Southwestern Oklahoma State University, Weatherford, OK, USA

Accepted for publication 22 May 2016
Correspondence: A. D. Farris, Arthritis and Clinical Immunology Program, OMRF, 825 NE 13th Street, Oklahoma City, OK 73104, USA.
E-mail: darise-farris@omrf.org

[1]These authors contributed equally to this work.

Summary

RNA-binding nuclear antigens are a major class of self-antigen to which immune tolerance is lost in rheumatic diseases. Serological tolerance to one such antigen, La/Sjögren's syndrome (SS)-B (La), is controlled by $CD4^+$ T cells. This study investigated peripheral tolerance to human La (hLa) by tracking the fate of hLa-specific $CD4^+$ T cells expressing the transgenic (Tg) 3B5.8 T cell receptor (TCR) after adoptive transfer into lymphocyte-replete recipient mice expressing hLa as a neo-self-antigen. After initial antigen-specific cell division, hLa-specific donor $CD4^+$ T cells expressed forkhead box protein 3 (FoxP3). Donor cells retrieved from hLa Tg recipients displayed impaired proliferation and secreted interleukin (IL)−10 *in vitro* in response to antigenic stimulation. Transfer of highly purified FoxP3-negative donor cells demonstrated that accumulation of hLa-specific regulatory T cells (T_{reg}) was due primarily to expansion of small numbers of donor T_{reg}. Depletion of recipient plasmacytoid dendritic cells (pDC), but not B cells, severely hampered the accumulation of $FoxP3^+$ donor T_{reg} in hLa Tg recipients. Recipient pDC expressed tolerogenic markers and higher levels of co-stimulatory and co-inhibitory molecules than B cells. Adoptive transfer of hLa peptide-loaded pDC into mice lacking expression of hLa recapitulated the accumulation of hLa-specific T_{reg}. Blockade of the type 1 interferon (IFN) receptor in hLa Tg recipients of hLa-specific T cells impaired $FoxP3^+$ donor T cell accumulation. Therefore, peripheral expansion of T_{reg} specific for an RNA-binding nuclear antigen is mediated by antigen-presenting pDC in a type 1 IFN-dependent manner. These results reveal a regulatory function of pDC in controlling autoreactivity to RNA-binding nuclear antigens.

Keywords: La/SS-B, plasmacytoid dendritic cell, regulatory T cell, type 1 interferon

Introduction

Ubiquitous nuclear and cytoplasmic self-antigens that constitutively bind RNA are targeted selectively by the autoimmune response in several rheumatic diseases. Autoantibodies directed to one such antigen, La/Sjögren's syndrome (SS)-B, occur at high prevalence in SS [1] and mothers of infants with congenital heart block or neonatal lupus syndrome [2], and at lower prevalence in systemic lupus erythematosus (SLE) [3]. La-targeting antibodies co-occur typically with antibodies specific for Ro/SS-A [3], associate with a higher frequency of extraglandular manifestations of SS including vasculitis, leukopenia, hypergammaglobulinaemia, restrictive pulmonary disease and internal organ involvement [4–6] and have been reported to associate selectively with vasculitis in SLE [7]. Deciphering mechanisms of normal immunological tolerance to RNA-binding nuclear antigens is essential for understanding the development of autoimmunity to such antigens and for designing effective therapies for mitigating the disorders in which they occur.

Anti-La autoantibody production is T helper cell-dependent, as suggested by the association with specific class II human leucocyte antigen (HLA) alleles [8], class-switched isotypes [9], somatic hypermutation [9] and high serum concentration [10]. Using mice expressing transgenic (Tg) human La (hLa) with ubiquitous, nuclear

 © 2016 British Society for Immunology,

distribution at physiological levels, we have previously reported detectable immunological tolerance in hLa-specific $CD4^+$ T cells, but not in B lymphocytes [11,12]. Further studies revealed that human La-specific T cells in double Tg mice for an I-E^k-restricted T cell receptor (TCR) specific for hLa 67–76 peptide and the hLa neo-self-antigen undergo drastic but incomplete thymic clonal deletion [13]. A portion of residual, hLa-specific thymocytes in these mice developed into natural thymic regulatory T cells (T_{reg}), and the mice displayed high proportions of forkhead box P3 (FoxP3)-expressing, hLa-specific $CD4^+$ T cells in peripheral lymphoid organs that functioned to maintain immunological self-tolerance and prevent autoimmune lung pathology. However, it was possible that the lymphopenic environment caused by extensive thymic clonal deletion in this TCR/neo-self-antigen double Tg model was responsible for the increased proportion of T_{reg} in the periphery of these mice.

$CD4^+FoxP3^+$ T_{reg} are important for controlling autoimmunity and can develop in response to self-antigen in the thymus [14] or in the periphery from mature, naive T cells that have completed T cell development [15,16]. These cells suppress the responses of conventional T cells by several mechanisms, including immunosuppressive cytokines such as interleukin (IL)−10 or transforming growth factor (TGF)-β and various cell contact-dependent mechanisms [17]. Both types of cells contribute to the circulating T_{reg} pool, where they are regulated by homeostatic factors including IL-2 [18,19], TCR stimulation [20,21] and co-stimulation [22]. TCR and co-stimulatory signals promoting maintenance or conversion of T_{reg} in the periphery have been shown to be delivered by dendritic cells (DC) [23,24] and, in some instances, B lymphocytes [25–27], but the mechanisms involved are incompletely understood. DC subsets that have been implicated in peripheral T_{reg} induction in settings of transplantation, low dose peptide-induced tolerance and the steady state include $CD103^+$ gut DC [28], migratory dermal DC [29] and plasmacytoid pDC (pDC) [30–33]. pDC may drive T_{reg} development and expansion through low levels of antigen presentation and have been shown to promote T_{reg} through production of indoleamine-2,3-dioxygenase (IDO), co-stimulatory molecules, secretion of granzyme B and production of retinoic acid [34,35]. Existing studies have not addressed mechanisms of induction of T_{reg} specific for ubiquitous, RNA-binding nuclear antigens often targeted in systemic rheumatic diseases.

In the present study, we assessed the fate of hLa-specific T cells introduced into the periphery of lymphocyte-replete mice expressing the hLa neo-self-antigen in order to understand peripheral T_{reg} induction and homeostasis in T cells specific for a representative RNA-binding human nuclear autoantigen. We show that pDC induce expansion of hLa-specific $FoxP3^+$ T_{reg} in a type I interferon (IFN)-dependent manner. These studies uncover a regulatory mechanism that should be considered in terms of prophylactic and therapeutic approaches for systemic rheumatic diseases that employ type I IFN blockade.

Materials and methods

Mice

C57BL/6 (B6) mice congenic for H-2^k (B6.AK-$H2^k$/FlaEgJ; Jackson Laboratory, Bar Harbor, ME, USA) were crossed to B6 mice congenic for Thy1.1 (B6.PL-*Thy1*^{a}/CyJ; Jackson Laboratory) to generate B6.*H-2*$^{k/k}$.*Thy1.1*$^{+/+}$ mice. Line 3 hLa Tg mice described previously [11] were back-crossed to B6 mice at least 12 generations and crossed to B6.*H-2*$^{k/k}$.*Thy1.1*$^{+/+}$ congenic mice to generate heterozygous hLa transgenic (hLa Tg) or non-Tg H-$2^{k/k}$ Thy1.$1^{+/+}$ and H-$2^{k/b}$ Thy1.$1^{+/+}$ recipient mice. 3B5.8^+ hLa-specific TCR Tg mice described previously [13] were crossed to *Tcra*$^{-/-}$ (B6.129S2-*Tcra*^{tm1Mom}/J; Jackson Laboratory) and B6.H-2^k congenic mice to generate B6.*3B5.8*$^{+/-}$.*Tcra*$^{-/-}$.*H-2*$^{k/k}$ and B6.*3B5.8*$^{+/-}$*Tcra*$^{-/-}$.*H-2*$^{k/b}$ donor mice that are naturally Thy1.2^+. In addition, these donor mice were crossed with C57BL/6-*FoxP3*^{tm1Flv}/J mice (Jackson Laboratory), which express monomeric red fluorescent protein (mRFP)-tagged FoxP3, to generate B6.*3B5.8*$^{+/-}$.*Tcra*$^{-/-}$.*H-2*$^{k/k}$.*FoxP3-mRFP*$^+$ donor mice that are naturally Thy1.2^+.

Animals were maintained under specific pathogen-free barrier conditions in the OMRF Laboratory Animal Resource Center until experiments were carried out at 5–12 weeks of age. All studies were approved by the Oklahoma Medical Research Foundation Institutional Animal Care and Use committee.

Cell preparation

Splenocyte suspensions were obtained by passing spleens through 40 μm nylon filters, treating with Tris ammonium chloride solution (TAC; 0·14 M NH_4Cl in 17 mM Tris, pH 7·2) to lyse red blood cells and washing in Dulbecco's modified Eagle's medium (Sigma-Aldrich, Inc., St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS), 1× non-essential amino acids (Life Technologies, Grand Island, NY, USA), 2 mM L-glutamine, 10 μg/ml penicillin/streptomycin, 50 μM β-mercaptoethanol and 2 mM sodium pyruvate. Cells from lymph nodes were obtained similarly but without TAC treatment. Cells were quantified using trypan blue exclusion.

Bulk $CD4^+$ T cells and sorted T_{reg} were obtained from B6.*3B5.8*$^{+/-}$.*Tcra*$^{-/-}$.*H-2*$^{k/k}$.*FoxP3-mRFP* donor mice by pre-purification using CD4 (L3T4) MicroBeads (Miltenyi Biotec, San Diego, CA, USA) positive selection according to the manufacturer's protocol, followed by sorting of $mRFP^-$ ($FoxP3^-$) cells on an Influx cell sorter (BD Biosciences, San Diego, CA, USA).

To obtain adequate numbers of conventional DC (cDC) and pDC, mice were implanted with 5×10^6 Fms-like

kinase 3 ligand (Flt3L)-secreting B16 melanoma cells by subcutaneous injection to induce DC expansion [36]. Within 2 weeks of implantation, spleens were harvested, diced and digested at 37°C for 45 min in 10 ml RPMI-1640 supplemented with 10% FCS, 5 mM ethylenediamine tetra-acetic acid (EDTA), 15 mM HEPES, 1 mg/ml collagenase D (Sigma-Aldrich) and 0·1mg/mL DNase I (Roche Life Sciences, Indianapolis, IN, USA) followed by TAC treatment. $CD11c^+$ cells were enriched using CD11c MicroBeads (Miltenyi Biotec), according to the manufacturer's instructions. cDC and pDC were sorted further using antibodies directed to CD11c, CD19, CD317/PDCA1 and CD45R/B220 on a high-speed MoFlo XDP1 cytometer (Beckman Coulter, Brea, CA, USA) at > 98% purity.

Donor T cells retrieved from recipient spleens for *in-vitro* experiments were selected positively using anti-Thy1.2 MicroBeads (Miltenyi Biotec) and further purified by MoFlo, sorting for $Thy1.2^+$ $Thy1.1^-$ $CD4^+$ $V\beta10^+$ donor cells. The T cell-depleted fraction was irradiated (2200 rads) and used as antigen-presenting cells (APC).

Adoptive transfer experiments

For T cell transfers, 4–6 × 10^7 unlabelled or 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; Life Technologies, Grand Island, NY, USA)-labelled total splenocytes, 6 × 10^6 CFSE-labelled $CD4^+$ T cells or 2·5 × 10^6 $CD4^+$ $mRFP^-$ ($FoxP3^-$) cells harvested from B6.*3B5.8*$^{+/-}$.*Tcra*$^{-/-}$.*H-2*$^{k/k}$ or B6.*3B5.8*$^{+/-}$.*Tcra*$^{-/-}$.*H-2*$^{k/b}$ donor mice were resuspended in 0·2 ml phosphate-buffered saline (PBS) and injected retro-orbitally into hLa Tg or non-Tg H-$2^{k/k}$ $Thy1.1^{+/+}$ or H-$2^{k/b}$ $Thy1.1^{+/+}$ recipient mice, respectively. Seven days later (unless noted otherwise), spleens and lymph nodes from recipient mice were harvested and analysed by flow cytometry to assess cell proliferation, T_{reg} accumulation and B cell and DC phenotype.

Recipient pDC depletion was carried out by treating recipient mice with intraperitoneal (i.p.) injections of 400 μg anti-pDC antibody (120G8.04; Imgenex; Novus Biologicals, Littleton, CO, USA) [37] or immunoglobulin (Ig)G isotype control (BioXCell, West Lebanon, NH, USA) every 48 h beginning 2 days prior to cell transfer. B cell depletion was carried out by i.p. injection of 250 μg anti-CD20 antibody (MB20-11; generously provided by Dr Thomas Tedder) or IgG isotype control antibody (BioXCell) every 48 h beginning 1 day prior to cell transfer. To block the type I IFN receptor, recipient mice were treated i.p. with anti-IFN-alpha/beta receptor alpha chain (IFNAR1) [38] [monoclonal antibody mouse IFNAR1 (MAR1)−5A3; Leinco Technologies, St Louis, MO, USA] or IgG1 isotype control antibody (BioXCell). Antibody dosage began daily at 500 μg for the first 3 days (beginning 1 day prior to cell transfer) followed by 250 μg every 48 h thereafter until completion of the experiment.

Sorted splenic pDC were pulsed for 2 h with 10 μM hLa 61–84 peptide or hen egg-white lysozyme (HEL) 46–61 peptide in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 1× non-essential amino acids, 2 mM L-glutamine, 10 μg/ml penicillin/streptomycin, 50 μM β-mercaptoethanol and 2 mM sodium pyruvate at 1 × 10^6 cells/ml, washed twice and transferred subsequently (2 × 10^5 cells/recipient) into non-Tg H-$2^{k/k}$ $Thy1.1^+$ recipient mice. The next day, $3B5.8^+$ donor splenocytes were transferred into the same recipient mice. Euthanasia and cell retrieval were conducted 7 days post-3B5.8 T cell transfer.

Flow cytometry

Single-cell suspensions were treated with Fc block (anti-CD16/32 antibodies) for 5 min on ice, then stained with combinations of the following fluorophore-conjugated monoclonal antibody clones: CD4 (RM4-5), Vβ10 (B21.5), CD90.1(OX-7), CD90.2 (53-2.1), CD45R/B220 (RA3-6B2), CD11c (N418), CD19 (1D3), CD25 (PC61.5), CD86(GL1), CD80(16-10A1), MHC II(14-4-4S), CD200(OX-90), CD9 (KMC8), CCR9 (242503), pDC/IPC (120G8.04), FoxP3 (FJK-16s), PDCA-1(JF05-1C2.4.1), B7-DC (TY25) and B7-H1 (MIH5). FoxP3 staining was performed using a commercial kit (FoxP3 Staining Kit; eBiosciences, San Diego, CA, USA), according to the manufacturer's instructions. Data were collected on an LSRII cytometer (BD Biosciences, San Diego, CA, USA) and analysed with FACSDiva (BD Biosciences) or FlowJo (Treestar, Ashland, OR, USA) software.

In-vitro assays

Antibodies directed to the hLa antigen were measured by enzyme-linked immunosorbent assay (ELISA) as described previously [11] from sera of recipient mice up to 20 days after adoptive transfer of 3B5.8 cells.

In-vitro proliferation assays were performed as described previously with slight modifications [39]. Briefly, B6.*3B5.8*$^-$.*H-2*$^{k/k}$.*Tcra*$^{-/-}$ donor cells were transferred into hLa Tg and non-Tg mice as described above. Seven days post-transfer, donor $CD4^+$ T cells were isolated from recipients by positive selection with anti-Thy1.2 MicroBeads followed by cell sorting as described above. T cell depleted splenocytes were irradiated (2200 rads) and used as APC. Co-cultures containing a 1 : 4 T : APC ratio in the presence of 3·7 μM hLa 61–84 peptide were conducted for 72 h; 1 μCurie/well of [^{3}H]-thymidine was added for the last 18 h. Cultures were harvested onto glass fibre filters and counted by liquid scintillation. Supernatant from cell cultures was obtained at 48 h in order to measure IL-10 (Invitrogen, now Life Technologies) and TGF-β (eBiosciences, San Diego, CA, USA) by ELISA according to the manufacturers' protocols.

To determine DC antigen presentation capacity, cDC and pDC were purified from Flt3L-induced Tg or non-Tg mouse spleens as detailed above. Varying numbers of DC were cultured with 1 × 10^5 3C5.5 hLa 61-84-specific T cell hybridoma cells [40] or 3A9 HEL 46–61-specific 3A9

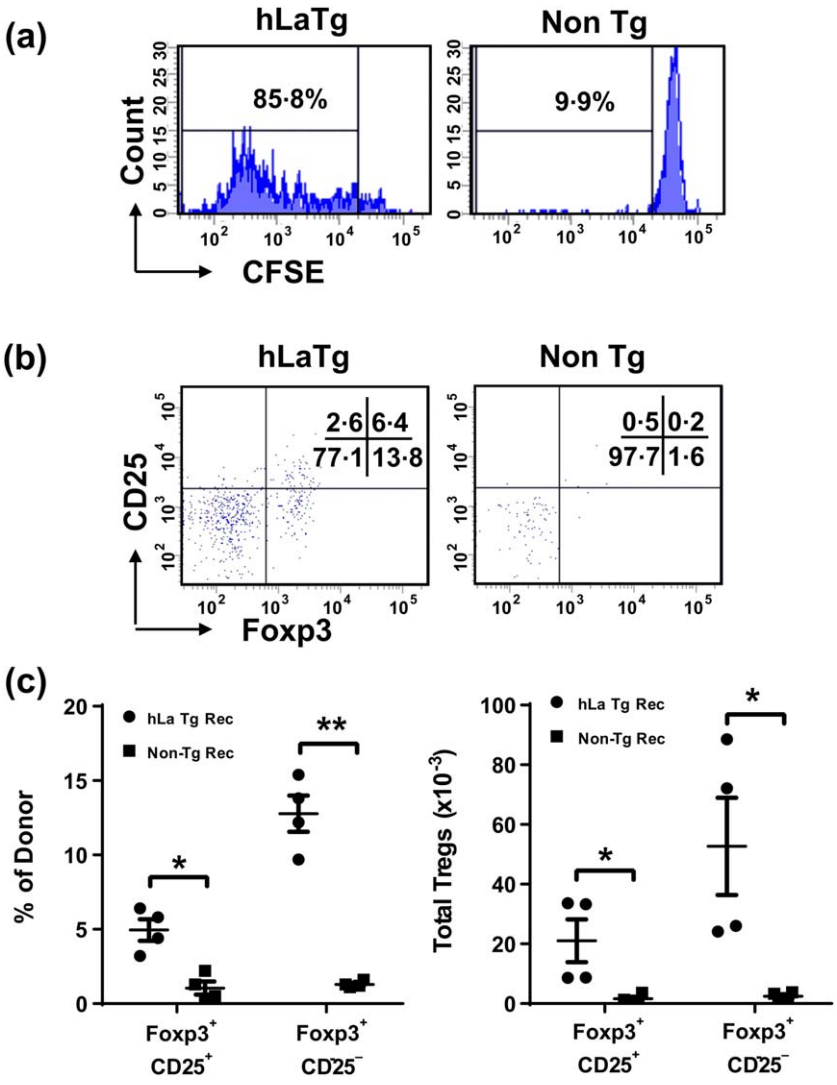

Fig. 1. Anti-human La (hLa)-specific 3B5.8 T cells differentiate into forkhead box protein 3 (FoxP3)-expressing cells in human La transgenic recipients. Representative (of four mice/group) flow cytometry plots depicting 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution (a) and regulatory T cell (T_{reg}) gating (b). Numbers in dot-plots refer to quadrant percentages. (c) Graphical representation of T_{reg} as a frequency of the total donor cell population (left) or absolute cell numbers (right) obtained from individual recipient mice (four mice/group). Statistics calculated by Student's *t*-test. $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

hybridoma cells [41] in V-bottomed 96-well plates for 24 h. Culture supernatants were collected to measure IL-2 production by ELISA (OptEIA ELISA Set; BD Biosciences).

Statistical analysis

Pairwise comparisons were evaluated using Student's *t*-test. *P*-values less than 0·05 were considered significant.

Results

The ubiquitous nuclear hLa neo-self-antigen induces expansion of antigen-specific T_{reg} in the periphery of lymphocyte replete mice

To assess the post-thymic fate of T cells specific for a representative RNA-binding nuclear antigen, CFSE-labelled 3B5.8 TCR^+ hLa-specific T cells from Thy 1.2^+ B6.*3B5.8*$^{+/-}$*H-2*$^{k/b}$*Tcra*$^{-/-}$ donor mice (referred to hereafter as 3B5.8 TCR Tg) were transferred adoptively into groups of H-$2^{k/b}$ Thy$1.1^{+/+}$ recipient mice expressing the hLa neo-self antigen (hereafter referred to as hLa Tg). Recipients were Tg for the complete human gene for La, including its natural promotor, and expressed nuclear-localized hLa ubiquitously at levels similar to the endogenous mouse La protein [11]. Donor cells were recovered from secondary lymphoid organs of recipient mice 7 days post-transfer and examined for proliferation status. As assessed by the fraction of cells diluting CFSE, donor $CD4^+$ T cells divided extensively in hLa Tg (median 84·9% $CFSE^{low}$ fraction, range 82·1–86.7%, $n = 4$) but not non-Tg (median 7·1%, range 7·1–12·6%, $n = 3$) recipients by the 7-day time point (Fig. 1a). Similar results were observed in H-$2^{k/k}$ recipients (Supporting information, Fig. S1).

The hLa Tg recipient mice produced low, but detectable, levels of anti-La antibodies 14 and 20 days post-transfer (Supporting information, Fig. S2); total body pathological examination 20 days post-transfer revealed no evidence of lymphoid cell infiltrates in any tissues or organs, and all blood cell counts were normal (data not shown). As these

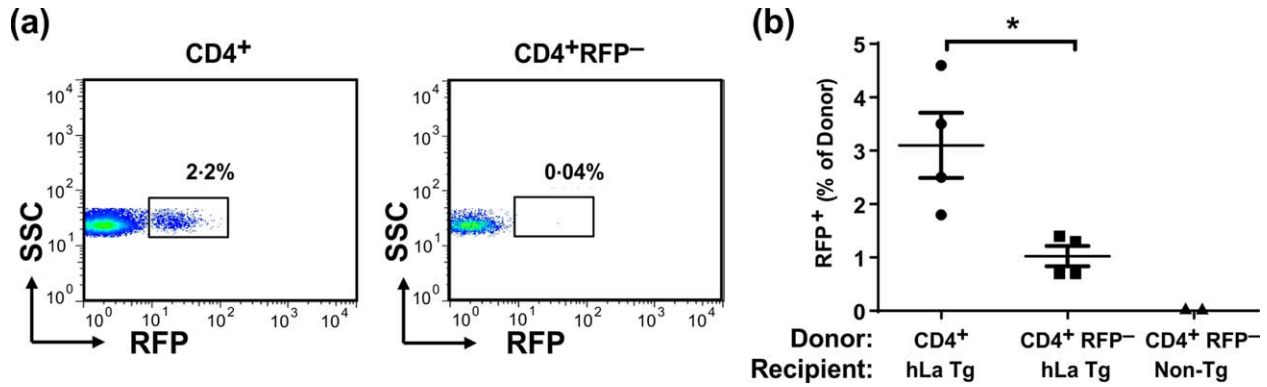

Fig. 2. hLa antigen-specific T_{reg} accumulation is due primarily to the expansion of pre-existing natural regulatory T cells (T_{reg}). (a) Post-sort dot-plots showing forkhead box protein 3 (FoxP3)-monomeric red fluorescent protein (mRFP) expression from total $CD4^+$ T cell sorts (left panel) and $CD4^+$ RFP^- cell sorts (right panel); (b) 7 days after adoptive transfer, donor T cells were analysed in individual recipient mice (four mice/group for hLa transgenic (Tg) recipients and two mice for non-Tg recipients) by flow cytometry for the frequency of FoxP3-mRFP-expressing T_{reg}. T_{reg} were observed in hLa-expressing spleens receiving either bulk $CD4^+$ cells or $CD4^+$ RFP^- cells from $3B5{\cdot}8^+$ donor mice. Statistics calculated by Student's *t*-test. $^*P < 0{\cdot}05$.

observations suggested potential control of autoreactivity by tolerogenic mechanisms, the potential development of peripheral T_{reg} in the model was evaluated. Donor splenocytes were transferred retro-orbitally into groups of hLa Tg and non-Tg recipient mice as discussed above. Seven days post-transfer, splenocytes and lymph node cells were recovered from recipients and analysed by flow cytometry for the expression of CD25 and the transcription factor, FoxP3. Increased frequencies and absolute numbers of donor cells with $CD25^+FoxP3^+$ and $CD25^-FoxP3^+$ T_{reg} phenotypes were recovered from hLa Tg recipient spleens (Fig. 1b,c) and lymph nodes (not shown) compared to those of non-Tg recipient mice. Similar results were observed in $H\text{-}2^{k/k}$ and $H\text{-}2^{k/b}$ recipient mice; thus, $H\text{-}2^{k/k}$ and $H\text{-}2^{k/b}$ transfer models were used interchangeably in subsequent experiments. In summary, 3B5.8 donor T cells recognize and respond to the hLa antigen as identified by cellular proliferation and accumulation of cells with a T_{reg} phenotype.

To determine if the accumulation of T_{reg} in this model arises from *de-novo* differentiation or marked expansion of small numbers of pre-existing T_{reg}, donor mice expressing a red fluorescent protein (RFP)-tagged FoxP3 locus [42] were employed. In this case total purified $CD4^+$ T cells, which included a small percentage of contaminating RFP^+ T_{reg} cells, or RFP^-CD4^+ ($FoxP3^-$) non-T_{reg} cells purified (at ~99% purity) from FoxP3-RFP donor mice (Fig. 2a) were transferred into recipient mice as described above. Spleen cells recovered from recipients 7 days post-transfer were analysed by flow cytometry for the frequency of donor RFP^+ ($FoxP3^+$) T cells. Both the bulk $CD4^+$ and highly purified RFP^-CD4^+ donor T cell populations were capable of generating RFP^+ T_{reg} following transfer into hLa Tg recipient mice, while RFP^+ cells were absent following transfer into non-Tg recipients (Fig. 2b). Furthermore, more extensive amplification of T_{reg} cells occurred in hLa Tg recipients that received bulk $CD4^+$ T cells compared to those that received sorted $CD4^+$ RFP^- non-T_{reg} cells (Fig. 2b). Therefore, the increased T_{reg} in hLa Tg recipients is due primarily to the expansion of pre-existing T_{reg}, although *de-novo* differentiation occurs to a lesser extent.

To examine further the characteristics of hLa-specific donor T cells after retrieval from hLa Tg mice, proliferation and production of immunosuppressive cytokines in response to *in-vitro* antigenic challenge were assessed. Donor cells retrieved from recipient mice 7 days post-transfer and challenged with hLa 61-84 peptide *in vitro* exhibited defective proliferation and secreted IL-10 (Fig. 3). TGF-β1 was undetectable under similar conditions (data not shown).

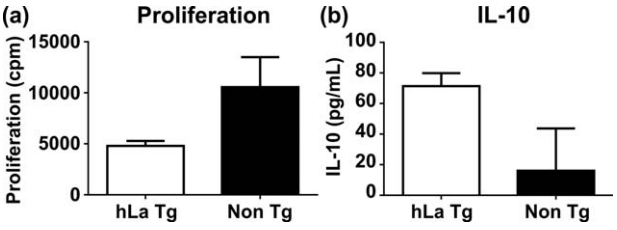

Fig. 3. 3B5.8 donor T cells retrieved from human La transgenic (Tg) recipients secrete interleukin (IL)-10 and are hypo-proliferative *in vitro*. Following adoptive transfer, donor T cells were purified from pooled (nine mice/group) recipient splenocytes and co-cultured with irradiated antigen-presenting cells (APC) in the presence of 3·7 μM of hLa 61–84 peptide for 72 h for measurement of cell proliferation by incorporation of [^{3}H]-thymidine (a) and 48 h for evaluation of IL-10 in supernatants by enzyme-linked immunosorbent assay (ELISA) (b). All values are expressed as mean ± standard deviation (s.d.) of triplicate wells and are representative of similar results from two independent experiments.

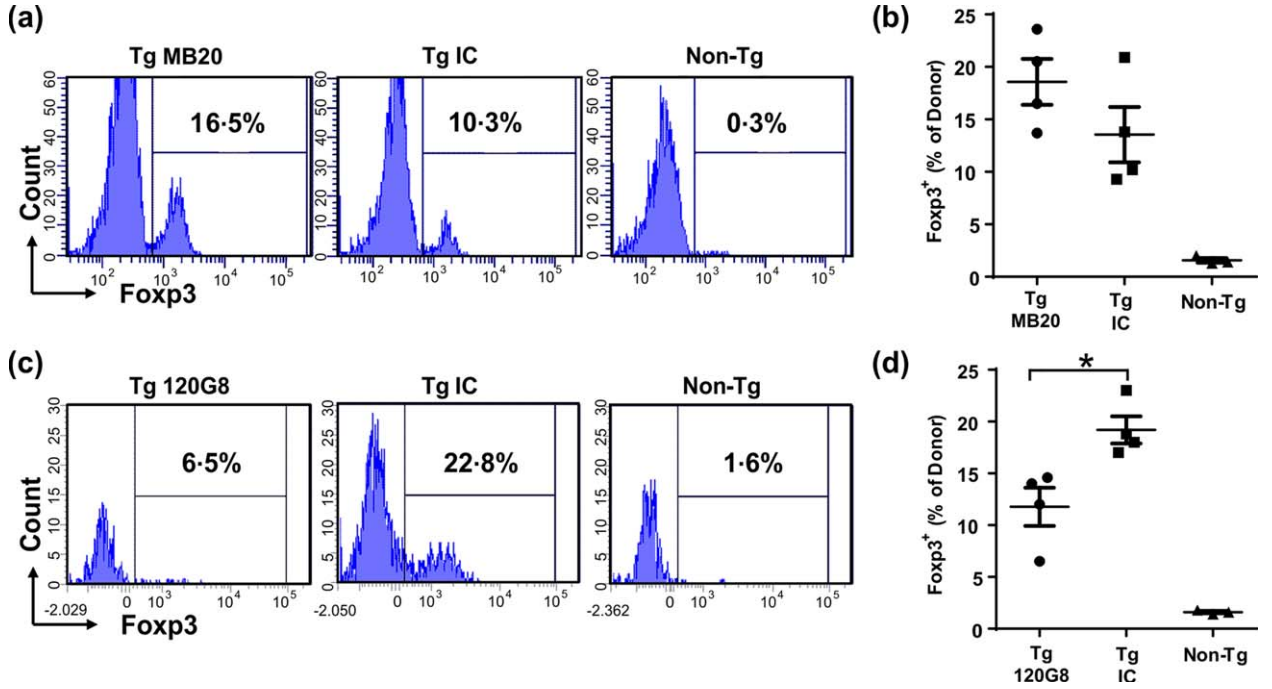

Fig. 4. Plasmacytoid dendritic cell (pDC) but not B cell depletion reduces regulatory T cell (T_{reg}) accumulation. (a,c) Representative (of three to four mice/group) histograms showing forkhead box protein 3 (FoxP3) expression among donor T cells transferred into B cell-depleted (MB20; (a) or pDC-depleted [120G8; (c)] hLa transgenic (Tg) (Tg; four mice/group) or non-Tg (three mice/group) recipient mice. (b,d) Frequencies of $FoxP3^+$ donor T cells 7 days post-transfer. Graphs show mean ± standard error of the mean (s.e.m.) of individual mice. Statistics were calculated by Student's *t*-test. $^*P < 0{\cdot}05$.

Plasmacytoid dendritic cells but not B lymphocytes promote expansion of hLa-specific T_{reg}

B lymphocytes and pDC have been implicated selectively in pathogenic responses to RNA-binding nuclear antigens such as La [43] and have also been shown to aid in the generation of peripheral T_{reg}. To determine if these cell types drive peripheral expansion of T_{reg} in the present model, recipient mice were depleted selectively of these cells throughout the duration of the experiment. Treatment of hLa Tg recipient mice with B cell- or pDC-depleting antibodies resulted in elimination of B lymphocytes (Supporting information, Fig. S3) or pDC (Supporting information, Fig. S4), respectively. B lymphocytes were not required for the increased appearance of donor-derived T_{reg} (Fig. 4a,b). In contrast, depletion of pDC impaired the development of hLa-specific T_{reg} in hLa Tg recipient mice significantly (Fig. 4c,d). Thus, pDC but not B lymphocytes promote peripheral expansion of T_{reg} specific for a ubiquitous, RNA-binding nuclear antigen.

To determine whether previously proposed tolerogenic (CD199/CCR9 [32], CD200 [44] and CD9 [45]), co-inhibitory (CD274/B7-H1/PDL-1 and CD273/B7-DC/PD-L2) or co-stimulatory (CD80 and CD86) markers were expressed selectively on pDC following adoptive transfer of hLa-specific donor cells, recipient pDC ($CD11c^+CD45R/B220^+CD317/120G8^+CD317/PDCA1^+$) and B lymphocytes ($CD45R/B220^+CD11c^-$), were characterized phenotypically 7 days post-transfer. Compared to B cells, pDC exhibited increased expression of MHC class II, CD199/CCR9, CD200, CD9, CD80 and CD86, as well CD274/B7-H1 and CD273/B7-DC (Fig. 5). Compared to cDC ($CD11c^+CD45R/B220^-CD317/120G8^-CD317/PDCA1^-$), pDC expressed lower levels of MHC class II and CD9 and increased expression of CD199/CCR9, CD200, CD274/B7-H1and CD273/B7-DC (Supporting information, Fig. S5).

hLa Tg pDC constitutively present the hLa 61-84 epitope and autonomously induce 3B5.8 T_{reg} *in vivo*

We have shown previously that hLa Tg B lymphocytes constitutively present hLa T cell epitopes [11]. To determine whether pDC and cDC constitutively present the hLa 61-84 epitope *in vivo* in hLa Tg recipient mice, pDC and cDC from both hLa Tg and non-Tg mice were sorted and co-cultured with an $I\text{-}E^k$-restricted hLa 61–84-specific T cell hybridoma in the absence of any exogenous hLa antigen. Following 24-h co-culture, IL-2 production was measured from cell culture supernatants. Compared to those from microcultures containing non-Tg DC, hLa-specific T cells from microcultures containing hLa Tg cDC and, to a lesser extent, hLa Tg pDC, secreted increasing IL-2 with increasing DC numbers (Fig. 6a), indicating constitutive presentation of the hLa 61–84 epitope by cDC and pDC in hLa Tg

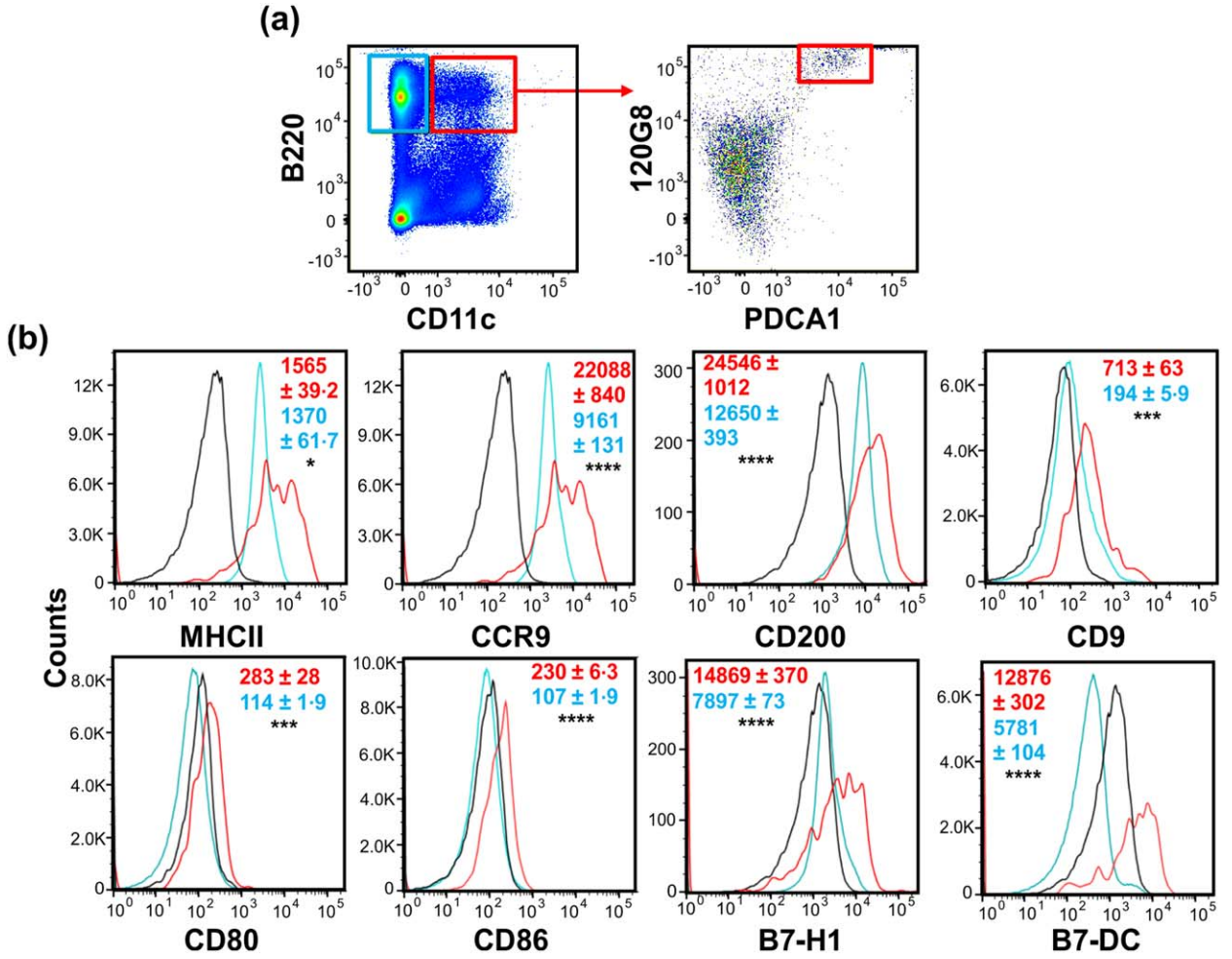

Fig. 5. Cell surface phenotype of plasmacytoid dendritic cells and B cells following T cell adoptive transfer. Following adoptive transfer, recipient splenocyte B cell and pDC populations were analysed for expression of surface molecules. (a) Gating strategies distinguishing B cells (blue box) from pDC (red boxes). (b) Representative (of four mice/group) histograms showing expression of major histocompatibility complex (MHC) class II (MHCII), CCR9, CD200 and CD9 (top) and co-stimulatory molecules CD80, CD86, B7-H1 and B7-DC (bottom). Numbers indicate mean fluorescence intensities (MFI) ± standard error of the mean (s.e.m.) of the respective cell surface proteins from individual mice (four mice/group), with text colours corresponding to flow cytometry gates depicted in (a). Statistics were calculated using Student's *t*-test. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; $^{****}P < 0{\cdot}0001$.

mice. In contrast, cDC and pDC isolated from hLa Tg mice failed to elicit responses from the 3A9 T cell hybridoma that is specific for an I-A^k-restricted epitope of the irrelevant HEL antigen.

To determine whether hLa peptide-loaded pDC alone from non-Tg mice could induce 3B5.8 T_{reg} expansion in the absence of endogenously expressed hLa antigen *in vivo*, highly purified pDC were loaded with either hLa 61–84 peptide or irrelevant control I-A^k-restricted HEL 46–61 peptide and transferred subsequently into non-Tg recipient mice. Peptide loading was confirmed by the capacity of peptide-loaded DC to specifically stimulate the relevant hybridomas (Supporting information, Fig. S6). The following day, cells from 3B5.8$^+$ donor mice were also transferred into recipient mice. Seven days after donor T cell transfer, spleens were harvested and donor 3B5.8 T_{reg} were assessed by flow cytometry as in earlier experiments. pDC loaded with hLa peptide, but not pDC loaded with control peptide, induced accumulation of 3B5.8 TCR-expressing T_{reg} (Fig. 6c,d). Thus, pDC not only present the hLa antigen constitutively in hLa Tg mice, but can also promote the generation of antigen-specific T_{reg} when provided as the sole hLa 61–84 peptide-presenting cell *in vivo*.

Type 1 IFN enhances hLa-specific T_{reg} expansion

pDC are specialized type I IFN-producing cells [43]. In the present model, pDC are also important mediators in promoting self-antigen-specific T_{reg}. To determine whether type I IFN contributes to the enhanced generation of hLa-specific T_{reg} in the periphery of hLa Tg mice, hLa Tg recipients were treated with a blocking monoclonal antibody

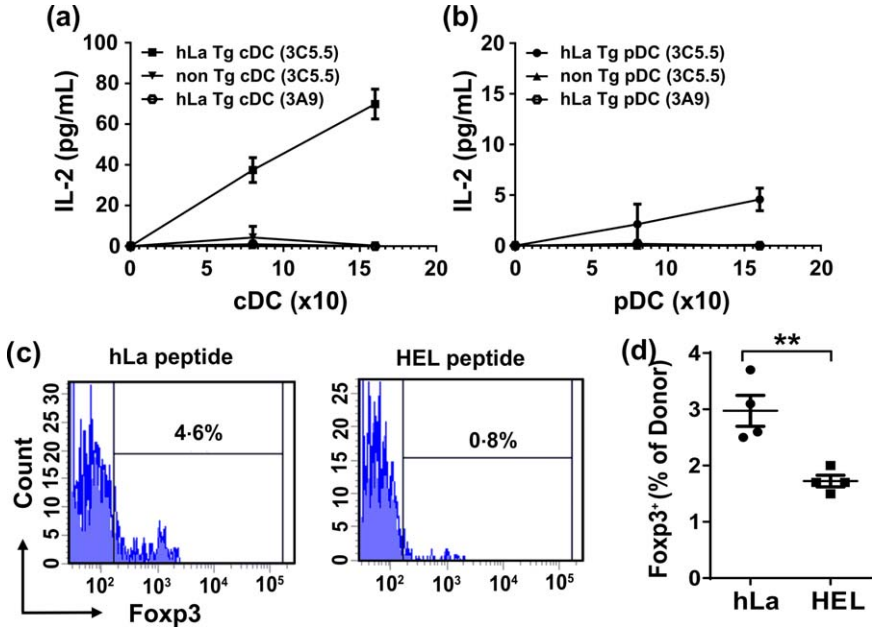

Fig. 6. Plasmacytoid dendritic cells constitutively present hLa antigen to T cells. (a,b) conventional DC (cDC) and pDC sorted from hLa-transgenic (Tg) and non-Tg mice were co-cultured with the hLa-specific 3C5.5 T cell hybridoma or the irrelevant hen egg-white lysozyme (HEL)-specific 3A9 hybridoma for 24 h as indicated. Interleukin (IL-2) in the supernatant was measured by enzyme-linked immunosorbent assay (ELISA). Values are the mean ± standard deviation (s.d.) of triplicate wells. Similar results were observed in three independent experiments.
(c) Peptide-pulsed non-Tg pDC and 3B5.8 splenocytes were transferred adoptively into non-Tg B6.*H-2*$^{k/k}$.*Thy1.1*$^{+/+}$ recipient mice on consecutive days. Seven days post-transfer, spleen cells were recovered from recipient mice for analysis of T_{reg} development. Representative (of four mice/group) histograms show forkhead box protein 3 (FoxP3) expression within donor T lymphocytes from mice receiving hLa peptide-loaded pDC (left) or HEL peptide-loaded pDC (right). (d) Frequencies of FoxP3-expressing T_{reg} cells from individual recipient mice (four mice/group) after adoptive transfer. Graphs show mean ± standard error of the mean (s.e.m.). Statistics were calculated by Student's *t*-test. $^{**}P < 0{\cdot}01$.

specific for the IFN-α/β (IFN-α/β) receptor, IFNAR1, throughout the T cell adoptive transfer experiment. Numbers of hLa-specific T_{reg} were reduced significantly among anti-IFNAR1 antibody-treated hLa Tg recipient mice compared to those that had been treated with an isotype control antibody (Fig. 7). Therefore, type I IFN promotes accumulation of donor hLa-specific FoxP3$^+$ regulatory T cells in the periphery of hLa Tg recipient mice.

Discussion

RNA-binding antigens are key targets of the autoimmune response in several rheumatic diseases, a phenomenon that is thought to arise from the capacity of RNA-binding nuclear antigen-containing immune complexes to stimulate RNA sensors in accessory cells such as B lymphocytes and pDC [43]. Mechanisms of normal immunological tolerance to this class of self-antigen have not been well studied. Moreover, although T_{reg} are recognized as critical for the control of autoimmune disease, mechanistic details of their regulation are still incomplete. We observed recently that thymic clonal deletion and development of thymic T_{reg} are important mechanisms of tolerance to the hLa neo-self-antigen that act to prevent high titres of anti-hLa autoantibodies, cellular autoimmunity and pulmonary pathology [13]. Herein we investigated the regulation of hLa antigen-specific T_{reg} in the periphery of mice using an adoptive transfer system.

The data presented show that a representative, ubiquitously expressed RNA-binding nuclear antigen can elicit antigen-specific T_{reg} in the periphery of mice. Antigen exposure without concurrent inflammation may promote conversion of FoxP3$^-$ T cells into FoxP3$^+$ T_{reg} [46]. Although there was evidence for *de-novo* T_{reg} differentiation in the hLa-specific donor T cell/hLa Tg recipient adoptive transfer model, the major effect was expansion of pre-existing T_{reg}. In the present study, donor T cells proliferated in recipient mice and induced low, but detectable, levels of autoantibodies directed to the hLa neo-self-antigen. Using adoptive transfer of hLa 61–84 peptide-primed polyclonal T cells, we established previously that non-tolerant donor cells can, at least transiently, elicit autoantibodies in hLa Tg recipient mice, while CD4$^+$ T cells from tolerant hLa Tg donor mice cannot [11]. The La autoantibodies observed in the present study were of lower titre at all time-points compared to the prior study, despite transfer of much higher numbers of hLa-specific donor T cells. We speculate that the low autoantibody levels and lack of autoimmune pathology following the transfer of large numbers of hLa-specific T cells from TCR Tg donor mice were limited by

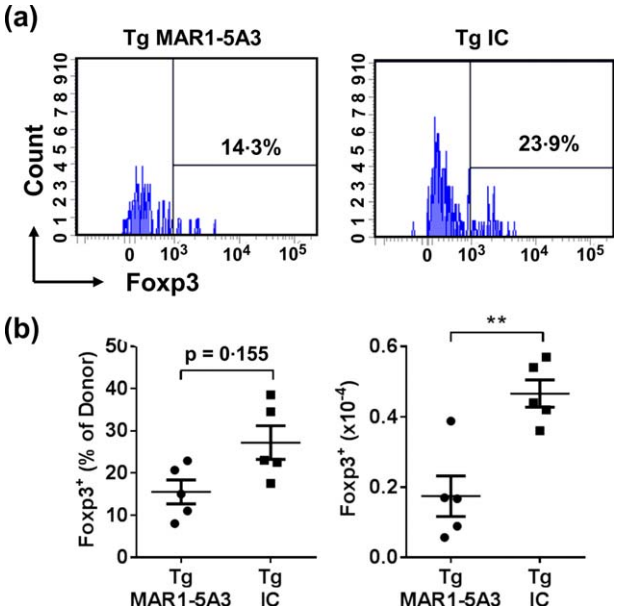

Fig. 7. Type I interferon (IFN) is important for induction of a forkhead box protein 3 ($FoxP3^+$) T_{reg} phenotype in hLa-specific 3B5.8 donor cells. Recipient mice were treated with the IFN alpha receptor 1 (IFNAR1) blocking monoclonal antibody mouse IFNAR1 (MAR1)-5A3 or a matched isotype control antibody before and during adoptive transfer. Seven days post-transfer, donor T cells were examined by flow cytometry to assess T_{reg} development. (a) Representative (of four mice/group) histograms of FoxP3 expression among donor T cells obtained from recipient mice treated with MAR1-5A3 antibody (left) or isotype control antibody (right). (b) Frequencies (left) and absolute numbers (right) of T_{reg} among donor T cells (four mice/group). Graphs show mean ± standard error of the mean (s.e.m.) of individual mice. Statistics were calculated by Student's *t*-test. $^{**}P < 0{\cdot}01$.

the observed expansion of T_{reg}. This thesis is supported by our recent study showing that impairment of thymic T_{reg} differentiation and T_{reg} function in a hLa/TCR double Tg mouse model led to high titre hLa autoantibody responses and subsequent autoimmune lung pathology [13]. Understanding the mechanisms of RNA-binding nuclear antigen-specific T_{reg} accrual may permit the design of rheumatic disease-targeted therapies that can preserve this beneficial regulatory response selectively while simultaneously curtailing pathogenic cell subsets.

The pDC and B lymphocyte subsets are major pathogenic cell types in lupus and related disorders such as SS and are the targets of existing or developing therapies [47,48]. The present studies show clearly that pDC but not B cells are required for efficient peripheral T_{reg} expansion, as their elimination in recipient mice hampered hLa-specific T_{reg} accumulation severely in the adoptive transfer model. The mechanism of pDC expansion involved antigen presentation, as hLa peptide-loaded pDC alone could elicit hLa-specific T_{reg} expansion *in vivo* in the absence of any other hLa antigen source. Moreover, pDC from hLa Tg recipient mice presented endogenous hLa peptide constitutively, but at a lower level than conventional DC as measured by the readout of hLa 61–84 peptide-specific hybridoma stimulation and by cell surface MHC class II levels. This is consistent with high rates of MHC class II turnover among activated pDC that keeps presentation of specific antigens low [49] and with induction of T_{reg} by low peptide concentrations [31].

Cell surface phenotyping of recipient T_{reg}-inducing pDC revealed expression of tolerogenic pDC markers reported by others, as well as enhanced expression of both co-stimulatory and co-inhibitory markers, all of which could contribute mechanistically to T_{reg} expansion. In particular, pDC in our model expressed high levels of CD199/CCR9, reported to be a marker for tolerogenic, immature DC that control graft-*versus*-host disease by enhancing T_{reg} function [32]. Tolerogenic pDC in our model also expressed CD200, which can induce T_{reg} through an IDO-dependent mechanism [44]. Interestingly, only activated pDC can secrete IDO [44]. Low levels of CD9 were reported previously to mark tolerogenic immature pDC that produced negligible levels of IFN-α, while $CD9^+$ pDC were immunostimulatory and secreted IFN-α in response to Toll-like receptor ligation [45]. In the present study, the tolerogenic pDC expressed CD9 levels that were intermediate between B cells and cDC, consistent with a phenotype permissive for IFN-α secretion. Although the role of immature DC in inducing T_{reg} is established, mature DC expressing high levels of co-stimulatory molecules can also expand T_{reg} preferentially [50,51]. Moreover, B7-H1 has been implicated in T_{reg} expansion in the settings of infection [52,53] and allergy [54]. A similar role for CD273/B7-DC can be hypothesized, as these two ligands share the same receptor. Taken together, the phenotype of tolerogenic pDC in hLa Tg recipient mice is consistent with recently activated immature pDC. Further studies will be required to explore systematically the relevance of individual surface markers to the expansion of T_{reg} specific for ubiquitous RNA-binding nuclear antigens.

Although elevated type 1 IFN levels are pathogenic in SLE and related disorders, we observed that the mechanism of expansion of T_{reg} specific for the representative RNA-binding nuclear antigen La was promoted by type 1 IFN. Thus, blockade of the type 1 IFN receptor IFNAR1 hampered T_{reg} accumulation of hLa-specific T cells severely after transfer into hLa Tg mice in this tolerogenic model. The present studies have not established the cellular source of T_{reg}-promoting type 1 IFN in the hLa adoptive transfer model; however, it is reasonable to speculate that the source may be pDC. CD200 can modulate type 1 IFN secretion by myeloid cells [55]. Thus, elevated CD200 expression and intermediate levels of CD9 [45] may mark pDC with the capacity to produce tolerogenic, non-pathogenic levels of type 1 IFN that promote T_{reg} accumulation. While the present paper was in preparation, Metidji and colleagues used IFNAR1-deficient mice to show that type 1 IFN promotes development of thymic T_{reg}

and survival of T_{reg} in the periphery of mice in a competitive or stress environment [56].

We conclude that pDC and type 1 IFN are important mechanisms controlling accumulation of RNA-binding nuclear antigen-specific T_{reg} in the periphery. These results inform prophylactic and therapeutic approaches for preventing and treating rheumatic autoimmune diseases wherein B cell depleting therapies may spare T_{reg}, while IFN-α blockade may inhibit not only pathogenic responses but also beneficial T_{reg} expansion.

Acknowledgements

The authors thank the Oklahoma Medical Research Foundation Flow Cytometry Core Facility, Laboratory Animal Resources Center, Imaging Core Facility and Graphics Resources Center for facilitating this work. The authors thank Dr. Thomas Tedder for generously providing the anti-CD20 mouse B cell depleting antibody. Funding was provided by the National Institutes of Health, USA [NIH R01 AI048097, P50 AR060804 and T32 AI007633 (C.G.H.)]. The contents are the sole responsibility of the authors and do not necessarily represent the official views of the NIH.

Disclosure

The authors have no competing interests to disclose.

Author contributions

A. D. F. and Z. P. designed the study. Z. P. and C. L. performed the experiments. Z. P., C. H. and A. D. F. analysed the data. C. H., Z. P. and A. D. F. wrote the paper.

References

1 Harley JB, Alexander EL, Bias WB *et al.* Anti-Ro (SS-A) and anti-La (SS-B) in patients with Sjogren's syndrome. Arthritis Rheum 1986; **29**:196–206.
2 Buyon JP, Winchester RJ, Slade SG *et al.* Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. Comparison of enzyme-linked immunosorbent assay and immunoblot for measurement of anti-SS-A/Ro and anti-SS-B/La antibodies. Arthritis Rheum 1993; **36**:1263–73.
3 Bruner BF, Guthridge JM, Lu R *et al.* Comparison of autoantibody specificities between traditional and bead-based assays in a large, diverse collection of patients with systemic lupus erythematosus and family members. Arthritis Rheum 2012; **64**:3677–86.
4 Pease CT, Charles PJ, Shattles W, Markwick J, Maini RN. Serological and immunogenetic markers of extraglandular primary Sjogren's syndrome. Br J Rheumatol 1993; **32**:574–7.
5 Martinez-Cordero E, Andrade-Ortega L, Martinez-Miranda E. Pulmonary function abnormalities in patients with primary Sjogren's syndrome. J Investig Allergol Clin Immunol 1993; **3**:205–9.
6 Locht H, Pelck R, Manthorpe R. Diagnostic and prognostic significance of measuring antibodies to alpha-fodrin compared to anti-Ro-52, anti-Ro-60, and anti-La in primary Sjogren's syndrome. J Rheumatol 2008; **35**:845–9.
7 Ramos-Casals M, Nardi N, Lagrutta M *et al.* Vasculitis in systemic lupus erythematosus: prevalence and clinical characteristics in 670 patients. Medicine (Balt) 2006; **85**:95–104.
8 Dudek NL, Maier S, Chen ZJ *et al.* T cell epitopes of the La/SSB autoantigen in humanized transgenic mice expressing the hLa class II haplotype DRB1*0301/DQB1*0201. Arthritis Rheum 2007; **56**:3387–98.
9 Raats JM, Roeffen WF, Litjens S *et al.* Human recombinant anti-La (SS-B) autoantibodies demonstrate the accumulation of phosphoserine-366-containing la isoforms in nucleoplasmic speckles. Eur J Cell Biol 2003; **82**:131–41.
10 Gordon TP, Greer M, Reynolds P, Guidolin A, McNeilage LJ. Estimation of amounts of anti-La(SS-B) antibody directed against immunodominant epitopes of the La(SS-B) autoantigen. Clin Exp Immunol 1991; **85**:402–6.
11 Keech CL, Farris AD, Beroukas D, Gordon TP, McCluskey J. Cognate T cell help is sufficient to trigger anti-nuclear autoantibodies in naive mice. J Immunol 2001; **166**:5826–34.
12 Aplin BD, Keech CL, de Kauwe AL, Gordon TP, Cavill D, McCluskey J. Tolerance through indifference: autoreactive B cells to the nuclear antigen La show no evidence of tolerance in a transgenic model. J Immunol 2003; **171**:5890–900.
13 Yaciuk JC, Pan YJ, Schwarz K *et al.* Defective selection of thymic regulatory T cells accompanies autoimmunity and pulmonary infiltrates in Tcra-deficient mice double transgenic for human La/Sjogren's syndrome-B and human La-specific TCR. J Immunol 2015; **194**:1514–22.
14 Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; **22**:531–62.
15 Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. Nat Rev Immunol 2003; **3**:253–7.
16 Daniel C, von Boehmer H. Extrathymic generation of regulatory T cells – chances and challenges for prevention of autoimmune disease. Adv Immunol 2011; **112**:177–213.
17 Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008; **8**:523–32.
18 Almeida AR, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J Immunol 2002; **169**:4850–60.
19 Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)−2 and induction of autoimmune disease by IL-2 neutralization. J Exp Med 2005; **201**:723–35.
20 Turner MS, Kane LP, Morel PA. Dominant role of antigen dose in CD4+Foxp3+ regulatory T cell induction and expansion. J Immunol 2009; **183**:4895–903.
21 Kim JK, Klinger M, Benjamin J *et al.* Impact of the TCR signal on regulatory T cell homeostasis, function, and trafficking. PLOS ONE 2009; **4**:e6580.
22 Zhang R, Huynh A, Whitcher G, Chang J, Maltzman JS, Turka LA. An obligate cell-intrinsic function for CD28 in Tregs. J Clin Invest 2013; **123**:580–93.
23 Darrasse-Jeze G, Deroubaix S, Mouquet H *et al.* Feedback control of regulatory T cell homeostasis by dendritic cells *in vivo*. J Exp Med 2009; **206**:1853–62.

24 Suffner J, Hochweller K, Kuhnle MC *et al.* Dendritic cells support homeostatic expansion of Foxp3+ regulatory T cells in Foxp3.LuciDTR mice. J Immunol 2010; **184**:1810–20.

25 Morlacchi S, Soldani C, Viola A, Sarukhan A. Self-antigen presentation by mouse B cells results in regulatory T-cell induction rather than anergy or clonal deletion. Blood 2011; **118**:984–91.

26 Sun JB, Xiang Z, Smith KG, Holmgren J. Important role for FcgammaRIIB on B lymphocytes for mucosal antigen-induced tolerance and Foxp3+ regulatory T cells. J Immunol 2013; **191**:4412–22.

27 Walters S, Webster KE, Sutherland A *et al.* Increased CD4+Foxp3+ T cells in BAFF-transgenic mice suppress T cell effector responses. J Immunol 2009; **182**:793–801.

28 Belkaid Y, Oldenhove G. Tuning microenvironments: induction of regulatory T cells by dendritic cells. Immunity 2008; **29**:362–71.

29 Azukizawa H, Dohler A, Kanazawa N *et al.* Steady state migratory RelB+ langerin+ dermal dendritic cells mediate peripheral induction of antigen-specific CD4+ CD25+ Foxp3+ regulatory T cells. Eur J Immunol 2011; **41**:1420–34.

30 Ochando JC, Homma C, Yang Y *et al.* Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. Nat Immunol 2006; **7**:652–62.

31 Kang HK, Liu M, Datta SK. Low-dose peptide tolerance therapy of lupus generates plasmacytoid dendritic cells that cause expansion of autoantigen-specific regulatory T cells and contraction of inflammatory Th17 cells. J Immunol 2007; **178**:7849–58.

32 Hadeiba H, Sato T, Habtezion A, Oderup C, Pan J, Butcher EC. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. Nat Immunol 2008; **9**:1253–60.

33 Huang Y, Bozulic LD, Miller T, Xu H, Hussain LR, Ildstad ST. CD8alpha+ plasmacytoid precursor DCs induce antigen-specific regulatory T cells that enhance HSC engraftment *in vivo*. Blood 2011; **117**:2494–505.

34 Swiecki M, Colonna M. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance. Immunol Rev 2010; **234**:142–62.

35 Lombardi V, Speak AO, Kerzerho J, Szely N, Akbari O. CD8alpha(+)beta(–) and CD8alpha(+)beta(+) plasmacytoid dendritic cells induce Foxp3(+) regulatory T cells and prevent the induction of airway hyper-reactivity. Mucosal Immunol 2012; **5**:432–43.

36 Vremec D, Segura E. The purification of large numbers of antigen presenting dendritic cells from mouse spleen. Methods Mol Biol 2013; **960**:327–50.

37 Asselin-Paturel C, Brizard G, Pin JJ, Briere F, Trinchieri G. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. J Immunol 2003; **171**:6466–77.

38 Sheehan KC, Lai KS, Dunn GP *et al.* Blocking monoclonal antibodies specific for mouse IFN-alpha/beta receptor subunit 1 (IFNAR-1) from mice immunized by *in vivo* hydrodynamic transfection. J Interferon Cytokine Res 2006; **26**:804–19.

39 Farris AD, Brown L, Reynolds P *et al.* Induction of autoimmunity by multivalent immunodominant and subdominant T cell determinants of La (SS-B). J Immunol 1999; **162**:3079–87.

40 Pan ZJ, Davis K, Maier SM *et al.* Neo-epitopes are required for immunogenicity of the La/SS-B nuclear antigen in the context of late apoptotic cells. Clin Exp Immunol 2006; **143**:237–48.

41 Allen PM, Unanue ER. Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. J Immunol 1984; **132**:1077–9.

42 Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proc Natl Acad Sci USA 2005; **102**:5126–31.

43 Pascual V, Farkas L, Banchereau J. Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol 2006; **18**:676–82.

44 Holmannova D, Kolackova M, Kondelkova K, Kunes P, Krejsek J, Andrys C. CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; Part I: CD200/CD200R structure, activation, and function. Acta Medica (Hradec Kralove) 2012; **55**:12–7.

45 Bjorck P, Leong HX, Engleman EG. Plasmacytoid dendritic cell dichotomy: identification of IFN-alpha producing cells as a phenotypically and functionally distinct subset. J Immunol 2011; **186**:1477–85.

46 Apostolou I, von Boehmer H. *In vivo* instruction of suppressor commitment in naive T cells. J Exp Med 2004; **199**:1401–8.

47 Cobo-Ibanez T, Loza-Santamaria E, Pego-Reigosa JM *et al.* Efficacy and safety of rituximab in the treatment of non-renal systemic lupus erythematosus: a systematic review. Semin Arthritis Rheum 2014; **44**:175–85.

48 Kirou KA, Gkrouzman E. Anti-interferon alpha treatment in SLE. Clin Immunol 2013; **148**:303–12.

49 Young LJ, Wilson NS, Schnorrer P *et al.* Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells. Nat Immunol 2008; **9**:1244–52.

50 Yamazaki S, Iyoda T, Tarbell K *et al.* Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 2003; **198**:235–47.

51 Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med 2004; **199**:1467–77.

52 Gupta N, Hegde P, Lecerf M *et al.* Japanese encephalitis virus expands regulatory T cells by increasing the expression of PD-L1 on dendritic cells. Eur J Immunol 2014; **44**:1363–74.

53 Rabe H, Nordstrom I, Andersson K, Lundell AC, Rudin A. Staphylococcus aureus convert neonatal conventional CD4(+) T cells into FOXP3(+) CD25(+) CD127(low) T cells via the PD-1/PD-L1 axis. Immunology 2014; **141**:467–81.

54 Gollwitzer ES, Saglani S, Trompette A *et al.* Lung microbiota promotes tolerance to allergens in neonates via PD-L1. Nat Med 2014; **20**:642–7.

55 Seeds RE, Mukhopadhyay S, Jones IM, Gordon S, Miller JL. The role of myeloid receptors on murine plasmacytoid dendritic cells in induction of type I interferon. Int Immunopharmacol 2011; **11**:794–801.

56 Metidji A, Rieder SA, Glass DD, Cremer I, Punkosdy GA, Shevach EM. IFN-alpha/beta receptor signaling promotes regulatory T cell development and function under stress conditions. J Immunol 2015; **194**:4265–76.

Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Fig. S1. hLa-specific T cells from 3B5.8 T cell receptor (TCR) transgenic (Tg) mice divide upon adoptive

transfer into hLa Tg recipient mice. 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE)-labelled 3B5.8 splenocytes from B6.3B5.8$^{+/-}$.H-2$^{k/k}$ donors were transferred retro-orbitally into B6.H-2$^{k/k}$ hLa Tg (four mice) or non-Tg (four mice) recipient mice. Recipients in this experiment were not congenic for Thy1.1. Splenocytes recovered from recipient mice 24 h, 48 h and 5 days (d) post-transfer were analysed by flow cytometry. Histograms show CFSE dilution of CD4$^+$Vβ10$^+$ cells (both unlabelled recipient cells and CFSE-labelled donor cells are visible). Percentages of undivided donor cells are shown.

Fig. S2. Anti-La immunoglobulin (Ig)G antibody titres from recipient mice at 0, 14 and 20 days following adoptive transfer of hLa-specific T cells. IgG antibody titres to (a) recombinant 6xhis-hLa or (b) irrelevant recombinant 6xhis-protective antigen (PA) from *Bacillus anthracis*. Each symbol represents the response of an individual mouse. Filled symbols represent hLa transgenic (Tg) recipients (three mice), open symbols represent non-Tg recipients (four mice).

Fig. S3. Treatment with anti-CD20 antibody (MB20) ablates CD19$^+$ cells. hLa transgenic (Tg) (four mice/group) or non-Tg (three) recipient mice were treated with B cell-depleting monoclonal antibody (MB20-11) or isotype control immunoglobulin (Ig)G prior to and during the duration of adoptive transfer. (a) Representative flow cytometry profiles of B cells from MB20-treated (left) or isotype control-treated (middle) hLa Tg recipient mice and untreated non-Tg recipient mice (right). (b) Frequencies of CD19$^+$ B cells from recipient mice. (c) Absolute numbers of CD19$^+$ B cells from recipient mice. Graphs show mean ± standard error of the mean (s.e.m) of individual mice. Statistics were calculated by Student's t-test. ****P $<$ 0·0001.

Fig. S4. Treatment with 120G8 antibody depletes plasmacytoid dendritic cells (pDC). hLa transgenic (Tg) (four mice/group) or non-Tg (three) recipient mice were treated with pDC-depleting monoclonal antibody 120G8 or isotype control immunoglobulin (Ig)G before and during adoptive transfer of donor cells. (a) Representative gating and flow profiles of pDC from 120G8 antibody treated (left) or isotype control treated (middle) hLa Tg recipients and untreated non-Tg recipients (right). Plots were gated on CD11c$^+$ cells. (b) Percentages of pDC from recipient mice. (c) Absolute numbers of pDC from recipient mice. Graphs show mean ± standard error of the mean (s.e.m) of individual mice. Statistics were calculated by Student's t-test. ****P $<$ 0·0001.

Fig. S5. Expression of cell surface molecules on recipient plasmacytoid dendritic cell (pDC) and conventional DC (cDC) populations following T cell adoptive transfer. Splenocytes were recovered from recipient mice 7 days post-transfer and analysed subsequently for cell surface markers. (a) Gating strategies distinguishing pDC (top; red box) from cDC (bottom; blue box). (b) Representative histograms showing expression of major histocompatibility complex (MHC) class II (MHCII), CCR9, CD200 and CD9 (top) and co-stimulatory molecules CD80, CD86, B7-H1 and B7-DC (bottom). Numbers indicate mean fluorescence intensity (MFI) of the respective cell surface proteins, where colours are representative of the flow cytometry gates depicted in (a). Cells were pre-gated on MHC class II$^+$ cells for the evaluation of MHC class II MFI values. Black line indicates isotype control from CD11c$^+$B220$^-$ cells. Statistics were calculated using Student's t-test. *P $<$ 0·05; **P $<$ 0·01; ***P $<$0·001; ****P $<$ 0·0001.

Fig. S6. Verification of peptide loading onto major histocompatibility complex (MHC) class II of plasmacytoid dendritic cells (pDC). Sorted pDC from Fms-like kinase 3 ligand (Flt3L)-induced wild-type B6 mice congenic for H-2^k (isolated from splenocytes pooled from two to three mice/group) were loaded with 10 μM of hLa 61–84 peptide (a) or hen egg-white lysozyme (HEL) 46–61 peptide (b) in vitro for 2 h, washed twice, then tested (8×10^3 peptide-loaded DC per well) for the capacity to stimulate the 3C5.5 hLa 61–84-specific T cell hybridoma or the 3A9 HEL 46–61-specific T cell hybridoma (1×10^5 hybridoma cells/well). In comparison, direct stimulation of 3C5.5 (c) and 3A9 (d) with peptides in the presence of irradiated splenic antigen-presenting cells (APC) (3×105 cells/well) demonstrates the greater inherent responsiveness of the 3C5.5 hybridoma compared to the 3A9 hybridoma for specific antigenic peptide. All results are the mean ± standard deviation (s.d.) of triplicate wells.