Human Tonsil-derived Dendritic Cells are Poor Inducers of T cell Immunity to Mucosally Encountered Pathogens

Claire M. Hallissey,¹ Robert S. Heyderman,¹,² and Neil A. Williams¹

¹School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom and ²Malawi-Liverpool-Wellcome Trust Clinical Research Programme, University of Malawi College of Medicine, Blantyre, Malawi

The mucosal immune system must initiate and regulate protective immunity, while balancing this immunity with tolerance to harmless antigens and bacterial commensals. We have explored the hypothesis that mucosal dendritic cells (DC) control the balance between regulation and immunity, by studying the responses of human tonsil-derived DC to Neisseria meningitidis as a model organism. We show that tonsil DC are able to sample their antigenic environment, internalizing Nm and expressing high levels of HLA-DR and CD86. However, in comparison to monocyte-derived DC (moDC), they respond to pathogen encounter with only low level cytokine production, largely dominated by TGFβ. Functionally, tonsil DC also only stimulated low levels of antigen-specific T cell proliferation and cytokine production when compared to moDC. We therefore propose that the default role for DC in the nasopharynx is to maintain tolerance/ignorance of the large volume of harmless antigens and bacterial commensals encountered at the nasopharyngeal mucosa.

Keywords. Dendritic cells; mucosal immunity; Neisseria meningitidis; tolerance; tonsils.

The nasopharyngeal mucosa is the site of colonization for numerous capsulated bacteria, including Neisseria meningitidis (Nm), which may invade to cause life-threatening disease [1, 2]. Typically, potential pathogens are carried harmlessly at the mucosal surface for a limited time and invasion does not occur or is contained by innate processes. However, under certain conditions bacteria cross the nasopharyngeal mucosa and cause systemic disease. The meningococcus, particularly serogroup B (MenB), represents a major health challenge in Europe and North America. Although there are MenB vaccines approaching licensure [3], their ability to provide broad protection and induce mucosal immunity is uncertain [4].

Dendritic cells (DC) are professional antigen presenting cells (APC) that internalise and process antigen for presentation to, and activation of, T lymphocytes. Various subtypes of DC have been identified and are believed to play different, although frequently overlapping, roles in controlling immune responses [5]. The type of T cell response initiated following pathogenic encounter can be directed via DC surface expression of various co-stimulatory/regulatory molecules, and secretion of immunomodulatory cytokines and chemokines [6, 7].

Interestingly, Nm has been shown to stimulate Th1 responses by driving production of IL-12 in monocyte-derived DC (moDC) [8–10]. Although a useful tool in cellular immunology, moDC do not fully represent tissue-derived DC. This is particularly true for mucosal DC, which exist in an antigen-rich mucosal environment and must balance induction of inflammatory Th1 responses against promotion of local antibody production via a Th2 phenotype, and/or induce regulatory activity to minimize immunopathological damage [11–13].

We have previously found that naturally-acquired human mucosal immune responses to MenB emerge with a Th1 bias, whereas peripheral blood T cell responses are
more balanced [14]. Our studies of responses from human tonsillar T cells also suggested that the Th1 response was balanced by the presence of meningococcal-specific regulatory T cells (Treg). Regulation dominates in teenagers, but T helper responses prevail by adulthood [15]. In this study we examine the role that nasopharyngeal DC play in either the eventual skewing of the mucosal immune response towards a Th1 type and/or the creation of meningococcal specific Treg. We isolated DC directly from the human nasopharynx and investigated their responses to pathogen-associated antigens.

**METHODS**

**Ethics Statement**
Palatine tonsils (PT) were obtained from consenting patients undergoing routine tonsillectomy for recurrent tonsillitis or airway obstruction at St Michael’s Hospital, Southmead Hospital or the Bristol Royal Hospital for Children, in Bristol, UK. No patient was suffering from tonsillitis at the time of operation. Patients with immunodeficiency or serious infections were excluded. The collection of tonsils and subsequent research comply with appropriate guidelines and institutional practices, and the study was approved by University of Bristol Hospital Trust Local Research Ethics Committee (reference E4388). Informed consent was obtained from participants or, for children, their legal guardians.

**Bacteria and Other Antigens**
*Neisseria meningitidis* strain H44/76 (B:15.P1.7,7,9) was used after overnight growth on horse brain heart infusion (HBHI) plates and fixation in 0.5% paraformaldehyde (PFA) in PBS. FITC-labelled PFA-fixed bacteria were created by incubation with 0.5 mg/mL FITC (Sigma Aldrich, Gillingham, UK) in PBS (30 minutes, 37°C). Dialysed inactive trivalent split virion influenza vaccine (Fluarix 2002/2003) was a control antigen. *E. coli* 0111:B4 LPS (Sigma-Aldrich) and CpG-ODN (InvivoGen, Berkeley, CA) were positive controls for DC activation.

**Antibodies for Flow Cytometry**
Fluorochrome-conjugated mouse monoclonal antibodies (mAb) were used in flow cytometry and for purification of tDC: CD19-PE, CD3-PE, CD123-PE, CD11c-PE, CD11c-APC (Miltenyi Biotec, Surrey, UK); CD14-PE, CD56-PE, CD16-PE (BD Biosciences, Oxford, UK); CD19-FITC, HLA-DR-PECy5.5, CD86-PE, CD80-PE, CD83-FITC (Invitrogen, Paisley, UK); CD123-PECy7, (eBioscience, Hatfield, UK); CD86-Pacific Blue (Cambridge Bioscience Ltd., Cambridge, UK).

**Preparation of moDC**
Peripheral blood mononuclear cells (PBMC) were isolated from buffy coat (National Blood Service, UK) via separation over Histopaque 1077 (Sigma-Aldrich). Monocytes were purified from PBMC using anti-CD14 MicroBeads (Miltenyi), according to manufacturer’s instructions. The monocytes were resuspended in complete RPMI 1640 (containing 100 U/mL penicillin, 0.1 mg/mL streptomycin, 4 mM L-glutamine (Invitrogen), and 10 mM HEPES (Sigma-Aldrich)) with 1000 U/mL rhGM-CSF and 500 U/mL rhIL-4 (Peprotech EC, London, UK), and incubated in 24-well plates for 6 days at 37°C. On days 2 and 4, cells were given fresh complete RPMI supplemented with rhGM-CSF and rhIL-4.

**Isolation of Mononuclear and Dendritic Cells From the Tonsil**
Tonsil mononuclear cells (TMNC) were isolated from PT using a method adapted from Davenport et al [14]. In brief, tonsils were cut into 2 mm³ pieces using a scalpel and dispersed through industrial steel mesh (Potter and Son, Bristol, UK). After washing with PBS, MNC were recovered from the tonsil cell suspension by separation on Histopaque 1077.

A DC-enriched TMNC fraction was isolated using a method adapted from Ruedl et al [16]. Tonsil MNC were resuspended in HBSS + 5 mM EDTA, then mixed with OptiPrep for a final iodixanol solution of density ρ = 1.085 g/mL. The sample was overlayed with 1.065 g/mL OptiPrep/RPMI solution, followed by HBSS. Following centrifugation at 400 × g for 15 minutes (20°C), DC-enriched cells were harvested from the top of the 1.065 g/mL iodixanol layer. Cells were then stained with PE-conjugated mAb against CD3, CD19, CD14, CD16 and CD56. For some experiments, antibodies against CD11c or CD123 were added. PE-stained cells were depleted using anti-PE magnetic MicroBeads (Miltenyi), according to the manufacturer’s instructions but with the addition of anti-CD19 beads to enhance B cell removal. Labelled cells were removed from the cell fraction using an LD column (Miltenyi).

**T cell Proliferation Assays**
TMNC or PBMC were enriched for T cells by using CD3 MicroBeads (Miltenyi), according to manufacturer’s instructions. Labelled cells were recovered using an LS Column (Miltenyi). T cell proliferative responses were monitored via incorporation of 0.4 μCi [3H]thymidine (Amersham Pharmacia Biotech, Little Chalfont, U.K.), as previously described [14]. Incorporation of tritiated thymidine was quantified using a Wallac MicroBeta TriLux liquid scintillation counter.

**Cytokine Analysis**
Levels of the cytokines IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, IFN-γ, TNF-α, and the chemokine MCP-1, were measured using Milliplex Human Cytokine Multiplex Immunoassay Kits (Millipore, USA) according to the manufacturer’s protocol. The assay plate was read on a Luminex 200 Total System (Luminex Corporation, Texas, USA), and data evaluated using Luminex xPONENT software. Levels of TGF-β in culture supernatant were measured using a Human TGF-b1
DuoSet ELISA Development System (R&D Systems Europe, Ltd. Abingdon, UK) according to the manufacturer’s protocol. The assay plate was read on an ELISA reader measuring absorbance at 450 nm, with reference 570 nm.

**Uptake of FITC-labelled Bacteria**

Dendritic cells in complete RPMI 1640 were incubated with FITC-labelled killed *N. meningitidis*, at a ratio of 1:100. At specified time points, cells were recovered by vigorous washing with refrigerated PBS, and analysed by flow cytometry or confocal microscopy. For flow cytometry, association of meningococci with DC was demonstrated using a method adapted from [17]. Cells were stained for expression of DC-associated surface markers and fixed in PFA prior to analysis. In some experiments, trypan blue (0.5 mg/mL) was added to quench extracellular FITC-fluorescence. For confocal microscopy, DC were incubated on Poly-L-lysine (Sigma-Aldrich) coated. Cells were fixed in 4% PFA, blocked with 5% BSA in PBS, and stained with non-conjugated anti-human HLA-DR mouse mAb (Invitrogen), followed by Alexa Fluor 594-conjugated goat anti-mouse IgG (Invitrogen). Stained cells were mounted using a glass cover slip and VECTASHIELD HardSet Mounting Medium with DAPI (Vector Laboratories, Peterbrough, UK), and imaged using a Leica AOBS SP2 confocal imaging system.

**Statistical Analysis**

Statistical analysis was carried out using GraphPad PRISM 6.03.

**RESULTS**

**Characterization of Tonsil DC**

The DC population isolated from adult human tonsils was characterized using their expression of key surface proteins previously identified as markers of DC-subtypes [5, 18, 19]. Purified cells did not have any PE fluorescence and so were negative for cell markers used in the purification process, which included CD14 for macrophage removal. Expression of HLA-DR was detected on 87%–98% of this enriched DC population, Approximately 85% of the HLA-DR+ population were CD123+ (Figure 1B) and designated as plasmacytoid DC (pDC); the minor CD11c+ population were designated as conventional DC (cDC). Compared to pDC, cDC had higher surface expression of HLA-DR and activation markers including CD83 and CD86 (Figure 1C).

Comparison of forward scatter-side scatter plots demonstrated that tDC were smaller and less granular than moDC (Figure 1A). Expression of CD11c was similar on cDC and moDC populations (Figure 1B), but expression of the pDC marker CD123 was low or

![Figure 1. Phenotypic analysis of tDC and moDC. A, Density plots show FSC/SSC profiles for the two cell types, with gates around the DC populations. B–C, Density plots and histograms show expression of indicated surface markers, following staining with fluorochrome-conjugated monoclonal antibodies. B, Data is shown for freshly isolated tDC and day 6 moDC. C, Data is shown for freshly isolated cells (dashed line) or following an overnight culture period with no antigenic stimulation (solid line). Shaded histograms are for unstained, fresh, cells. In the pDC and cDC plots the unstained cells are the whole tDC population, with no gating of CD11c+ or CD123+ populations. Data is representative of results from 3 similar experiments for each set of data, using cells obtained from 3 different subjects for tDC and 3 additional subjects for moDC.](https://academic.oup.com/jid/article-abstract/209/11/1847/2192883)
absent on moDC. Both tDC subsets upregulated surface expression of markers including HLA-DR, CD83 and CD86 after 18 hours of in vitro culture without exogenous antigen (Figure 1C). In contrast, freshly generated moDC had little change in surface marker expression after an additional 18 hours of culture.

Tonsil DC Stimulation of Antigen-specific T cell Proliferation

We examined the ability of tDC to stimulate antigen-specific T cell proliferation, by culturing both tDC and moDC with purified autologous T cells in the presence or absence of antigen. Proliferation of cultured cells was measured by tritiated

Figure 2. Comparison of the ability of moDC and tDC to stimulate antigen-specific T cell proliferation. Proliferation of the indicated cell populations was measured at various time points by pulsing with ³H thymidine for 18 hours. A. Collated data is shown for peak proliferation, measured over a 9 day culture, for TMNC and PBMC in the presence of H44/76 or influenza vaccine (Flu). Lines link data points from individual experiments. B. Data is shown for proliferation of purified T cells in the presence of PFA-fixed H44/76 (1 × 10⁸ cells/mL) (○) or influenza vaccine (90 ng hemagglutinin/mL) (△) and for T cells in co-culture with DC and H44/76 (●) or influenza (▲). Data is shown for 4 different subjects and is representative of experiments with cells from 4 subjects for blood T cells with moDC and 14 subjects for tonsil T cells with tDC. Results were similar regardless of the age of subjects. After optimization experiments to determine the cell concentrations which had the greatest proliferation, blood T cells were used at 0.25 × 10⁶ cells/mL, tonsil T cells were at 0.7 × 10⁶ cells/mL. The DC:T Cell ratio was 1:25 for moDC/blood T cells and 1:70 for tDC/tonsil T cells. Values plotted are means of 3 triplicate wells with error bars showing standard deviation across the wells.

C. Collated data is shown for peak antigen-dependent proliferation, measured on days 3 and 5, for tDC or moDC-stimulated T cells. Proliferation was measured for tonsil T cells in co-culture with tDC at a DC:T cell ratio of 1:50 (●) and 1:5 (○), and for blood T cells in co-culture with moDC at a ratio of 1:50 (▲). Stimulatory antigens were as indicated on the x-axis. Horizontal bars represent the median value for each data set. Significance of proliferation was assessed by comparison of thymidine incorporation in the presence and absence of antigen. *P<.05, **P<.01 (One way ANOVA with Dunnett’s multiple comparisons).

D. Data is shown for day 3 and 5 proliferation of tonsil T cells in co-culture with CD123 or CD11c-depleted tDC. Black bars are control cultures, white bars show thymidine incorporation due to proliferation in the presence of H44/76. T cells were at 5 × 10⁵ cells/mL, CD123- tDC were at a ratio of 1:10, and CD11c- tDC were at 1:5. Data is from individual experiments and is representative of results with cells taken from 4 subjects. Results were similar regardless of the age of subjects. Values plotted are means of 3 triplicate wells with error bars showing standard deviation across the wells. Data for all figures has been normalised by subtraction of the background level of thymidine incorporation by control cells cultured in medium alone, with no antigen. c.c.p.m – corrected counts per minute.
thymidine incorporation over a 9 day time course (Figure 2). As reported previously [14], whole TMNC and PBMC from most donors proliferated in response to both killed H44/76 MenB and influenza (Figure 2A). In all subjects where proliferative responses to both antigens occurred in whole PBMC cultures, moDC generated from the same donor stimulated proliferation of autologous T cells in response to these antigens (Figure 2B). At a similar DC:T cell ratio, tDC were poor stimulators of T cell proliferation, with responses being seen in only a minority of subjects. In the majority of donors (9 of 14) there was no T cell proliferation in response to either H44/76 or influenza.

Although there is increasing evidence that pDC can stimulate antigen-dependent activation and proliferation of T cells [20, 21], this major DC subset have historically been characterized as poor stimulators of antigen-dependent T cell proliferation in comparison to cDC. We therefore investigated whether tDC had the capacity to stimulate antigen-dependent T cell proliferation when at a higher DC:T cell ratio than necessary for moDC to stimulate proliferation. At a tDC:T cell ratio of 1:5, tDC stimulated T cell proliferation in response to influenza comparable to moDC at a ratio of 1:50 (Figure 2C). The tDC at this higher ratio also stimulated a significant level of antigen-dependent proliferation in response to H44/76.

Additional T cell proliferation assays were carried out using tDC depleted of either CD11c+ or CD123+ cells. Although individual data were variable, the tDC population enriched for pDC by removal of CD11c+ consistently stimulated less antigen-dependent T cell proliferation when compared with non-depleted tDC (Figure 2D). Similarly, removal of CD123+ DC consistently produced a cell population which stimulated greater T cell proliferation than untouched tDC but was still less stimulatory than moDC.

**Cytokine Production in Tonsil DC/T cell Cultures**

We used a Luminex assay to analyze cytokine production by DC-stimulated T cells, in order to generate a picture of the types of T cell response likely to be induced by tDC [22–24]. T cell cultures stimulated with H44/76 in the presence of moDC produced high levels of the proinflammatory/Th1-associated cytokines IFNγ and TNF-α (Figure 3A). In contrast, cultures of T cells with tDC produced much lower levels of cytokines. These responses were dominated by IL-17 and TGF-β, both of which were produced at similar levels in cultures without antigen stimulation. Figure 3B confirms that proliferation occurred in cultures from which supernatants were taken for cytokine analysis.

**Figure 3.** Analysis of the effects of H44/76 on cytokine secretion from T cells co-cultured with tDC and moDC. Purified T cells were cultured with DC for 5 days, in the presence of H44/76 (1 × 10^8 cells/mL). Proliferation was measured on days 3 and 5 via the incorporation of 3H thymidine, and cytokine levels were measured for supernatant samples taken on day 4. A, Data is shown for cytokine production by tonsil T cells in co-culture with tDC added at a 1:5 ratio, and for blood T cells in co-culture with moDC added at a 1:50 ratio. Black bars are control cells, white bars are cells in the presence of H44/76. Values plotted are mean concentrations from duplicate wells in the assay plate, with samples taken from a single experimental well in the co-culture plates. Error bars show standard deviation across the duplicates. B, Data is shown for proliferation of T cells within the same experiment as A. All data is from individual experiments and is representative of results using cells from 4 subjects. Results were similar regardless of the age of subjects.
Comparison of moDC and tDC Stimulation of T cell Proliferation

To address whether the poor tonsillar T cell responses were due to poor DC function, or impairment in the innate capacity of tonsillar T cells to respond to presented antigen, we collected blood samples from patients prior to tonsillectomy. This enabled us to investigate whether blood moDC were able to stimulate antigen-dependent responses in autologous tonsil T cells, and whether tDC were able to stimulate responses in autologous blood T cells. In the presence of meningococcal or influenza antigen, moDC stimulated similar levels of proliferation in T cells from tonsils and blood, indicating no difference in the capacity for responsiveness of T cell populations in the two tissues (Figure 4). Similarly, tDC were poor stimulators of both T cell responses (Figure 4).

Uptake of FITC-labelled Bacteria

To determine whether tDC have the ability to internalize antigen, we compared uptake of FITC-labelled H44/76 by moDC and tDC. Flow cytometric analysis of DC after a 20 hour culture with fluorescent H44/76 indicated that both tDC and moDC had a similar level of association with bacteria (Figure 5A). Two approaches were taken to verify that vesicular uptake of the associated bacteria had occurred. Firstly, trypan blue quenching was used to remove any fluorescence from externally attached bacteria [25]. The presence of a DC-associated fluorescent signal from bacteria after quenching established that internalization was taking place (Figure 5B). The levels of fluorescence were similar between tDC and moDC, suggesting a comparable endocytic capacity. The internalization of H44/76 was further confirmed by using confocal microscopy to visualize cells incubated with labelled bacteria (Figure 5C).

Staining with fluorochrome-conjugated anti-CD11c and anti-CD123 antibodies was used to distinguish the cDC and pDC populations respectively during flow cytometric analysis (Figure 5D). Association with bacteria occurred more rapidly in the cDC subset, but both tDC groups had similar levels of association at the end of a 20 hour culture period (Figure 5E).

Comparison of DC Responses to CpG DNA, Influenza and N. meningitidis

In order to investigate whether the poor capacity for T cell stimulation by tDC could be due to a failure to upregulate DC surface molecules involved in the interaction with T cells, we incubated tDC and moDC with various TLR agonists and used flow cytometry to measure surface expression of DC activation markers at different time points (Figure 6). After culture with H44/76, moDC had upregulated expression of HLA-DR as well as co-stimulation molecules including CD83 and CD86, but they did not upregulate expression of surface markers in response to influenza or CpG DNA. Tonsil DC expressed higher initial levels of HLA-DR and costimulatory markers than moDC, and stimulation with influenza or H44/76 antigens caused additional upregulation. In the case of influenza, upregulation of HLA-DR and CD86 was observed on cDC and HLA-DR on pDC. Exposure to H44/76 increased expression of CD86 on cDC. Predictably, CpG DNA primarily exerted effects on pDC, which are known to be high expressors of TLR9.

Comparison of Cytokines Produced by tDC and moDC in Response to Antigen

We compared levels of immunomodulatory cytokines in tDC and moDC cultures after overnight stimulation with antigen, using Luminex and ELISA analysis (Figure 7). Monocyte-derived DC produced a range of cytokines (IL-6, IL-10, IL-12p40, IL-12p70 and TNF-α) when cultured with H44/76. They also produced MCP-1 and TGF-β, both in the presence and absence of H44/76. Importantly, tDC produced undetectable or low levels of most cytokines examined. Supernatants from tDC cultures contained only low levels of IL-6, TNF-α and IL-10; and IL-12p40 and p70 were undetectable. Cultures of tDC were dominated by constitutively high levels of TGF-β.

DISCUSSION

The functional interaction of upper respiratory tract mucosal DC with colonizing bacteria has been largely unexplored. We have
shown here that, in comparison to moDC, tDC are poorly responsive to a variety of antigens. In addition to the limited capacity of tDC to stimulate antigen-specific T cell proliferation, cytokines released in co-cultures of tDC and tonsil T cells were at lower levels than cytokines produced in moDC/blood T cell cultures and had a different profile. Whereas moDC/T cell responses were dominated by high-level production of the Th1 cytokines TNF-α and IFNγ, tDC/T cell responses were dominated by TGFβ. Our failure to detect the Th2-associated cytokines IL-4 and IL-13 in tDC/T cell cultures supports previous work in the group, which detected Th1-dominated memory T cell responses in TMNC stimulated with meningococcal antigens [15]. Low level cytokine production and the absence of inflammatory TNF-α, along with high levels of constitutive TGFβ, in our tDC/T cell cultures could indicate that tDC are involved in induction of the Nm-specific Treg activity also identified in this previous study.

As DC undergo maturation, they lose their ability to take up and respond to newly encountered antigen [26]. Our data

Figure 5. Analysis of the uptake of FITC-labelled H44/76 by tDC and moDC. Purified tDC or moDC (5 × 10⁵ cells/mL) were cultured for up to 48 hours with FITC-labelled H44/76 (1 × 10⁸ cells/mL). The level of association of DC with MenB was assessed by flow cytometry and confocal microscopy. A, Data is shown for association of tDC and moDC with FITC-labelled bacteria after a 20 hour culture. Shaded histograms show the fluorescence of control cells. Values shown are the percentage of FITC positive DC. B, Data is shown for FITC fluorescence of tDC before (solid line) and after (dashed line) quenching of fluorescence due to external bacteria, via the addition of trypan blue (0.5 mg/mL). C, Images show the presence of internalized FITC-labelled H44/76 (green) within tDC and moDC. Cells were stained with a DAPI nuclear stain (blue), and an anti-surface HLA-DR antibody (red). D and E, Gating of the cDC and pDC populations within the tDC was carried out using staining with fluorochrome conjugated monoclonal antibodies against surface CD11c and CD123. D, Data is shown for association of CD11c+ and CD123+ tDC with FITC-labelled bacteria after a 20 hour culture. Shaded histograms show the fluorescence of control cells. E, Data is shown for CD11c+ (●) and CD123+ (▲) tDC over a 20 hour time course of incubation with FITC-labelled H44/76. Data points are median values from 3 experiments, error bars show standard deviation between experiments. ** indicates a significant difference in pDC and cDC proliferation (P< .01, 2-way ANOVA with Sidak multiple comparisons). Data in A–D is from individual experiments and is representative of results with cells from 3 subjects.
indicated that tDC were capable of internalizing antigen; but also that they matured rapidly in culture following isolation, even in the absence of antigen. Tissue DC isolated from other sites have been shown to mature and upregulate surface marker expression following isolation, yet retain the ability to stimulate strong T cell responses \[27, 28\]. It is therefore unlikely that the relative inability of tDC to stimulate T cells responses was due to the isolation procedure.

Studies of DC from mucosal sites including the gut and lungs have highlighted their role in maintaining immune homeostasis and minimizing inflammation, in addition to initiating protective immune responses. Mucosal DC in the gut appear particularly adapted to a more immunoregulatory role, and DC dysregulation is implicated in various diseases \[29, 30\]. Comparatively little information exists about the role mucosal DC might play in protection against disease caused by invasive pathogens within the nasopharynx. In this study we have explored how tDC might shape the immune response following encounters with potential pathogens, and help balance tolerance of nasopharyngeal commensals with establishment of protective immunity to potential pathogens. Although it is important to note that our studies use tonsils from patients undergoing tonsillectomy for either recurrent inflammation or obstruction, material from patients with active inflammation at the time of removal is excluded and over 10 years of using this material for studies of cellular immunity we have not noted consistent differences in levels of responses from these two sources.

Compared with moDC, the most notable deficit in tDC function was poor ability to produce cytokines that promote antigen-dependent T cell responses. The tDC constitutively secreted TGF-β at similar levels to moDC, but without the

Figure 6. Analysis of the effect of exposure to antigen on the surface marker expression of tDC and moDC. Purified tDC or moDC (5 × 10⁵ cells/mL) were incubated with influenza vaccine (90 ng hemagglutinin/mL), PFA-fixed H44/76 (1 × 10⁶ cells/mL) or CpG DNA (6 µg/mL). Data is shown for the expression of indicated surface markers at 6 hours (black bars), 18 hours (white bars) and 42 hours (grey bars). Values plotted are the median fluorescence intensity following staining with fluorochrome-conjugated antibodies. Data is from a single experiment with individual donors for the tDC and moDC, and is representative of results from 2–6 similar experiments using cells from different subjects (different total numbers of experiments were carried out for different antigens, time points and cell types). Results were similar regardless of the age of the subjects.
counteracting effect of the moDC’s inflammatory cytokine production to stimulate a Th1 immune response to antigen. The other cytokine secreted constitutively by tDC was IL-17, which might enhance mucosal immunity by aiding with the maintenance of mucosal barrier integrity [31]. The low level of tDC-derived cytokines in our studies suggests characteristics of ‘semi-mature’ DC, a recently described DC subset that have been associated with induction of Treg and T cell anergy.

The majority of antigen encountered in the nasopharynx is in the form of harmless environmental antigens or commensal bacteria. There would therefore be an evolutionary advantage to a default state of local APC which fails to stimulate T cell reactivity, or even promotes the development of tolerance. In keeping with this, we have shown that naturally acquired immunity to pneumococcal and meningococcal bacteria develops slowly over a number of years, and Th1 and Th17 responses are constrained by the induction of mucosa-associated Treg cells [15, 32]. It seems likely that the non-responsive tDC phenotype which we detected in this study might play a role in the development of these mucosal Treg. TGF-β plays a dual role in directing naïve T cell differentiation; promoting the generation of inducible Treg and, in concert with IL-6, driving differentiation of Th17 cells [33]. In culture, the suppressive effects of tDC-derived TGF-β may limit T cell proliferation; while in vivo its presence may eventually lead to the generation of Treg cells and a controlled level of Th17 responsiveness.

It seems likely that mechanisms might exist that enable tDC to acquire a more responsive and stimulatory phenotype to initiate T cell immunity during pathogenic invasion [34]. This would be in keeping with the phenotype of DC from mucosal sites such as the lung and intestine, and could be dependent on a change in function of the overall tDC population or activation of specific tDC subsets [30, 35–37]. We have found that the cDC subset of tDC are slightly more stimulatory than the pDC subset. Given the appropriate environment and stimulation, it could therefore be possible for at least a subset of tDC to initiate protective immune responses. Alternatively, immunity may depend on invasion of \textit{N. meningitidis} across the nasopharyngeal epithelium and encounter with local lymph node DC. The fact that Th1 responses can be stimulated in whole TMNC populations in vitro, suggests that once initiated, recall responses can be supported in the local tissue, potentially as a result of presentation by cognate memory B cells, which are abundant in the tonsils of adults.

Knowledge of the mechanisms involved in switching the DC role between maintenance of mucosal tolerance and initiation of inflammatory immunity will be important for enhanced understanding of host-pathogen interactions at the mucosa. Tonsil DC have the capability to control the balance between the establishment of commensal colonizations and the development of host immunity. This balance is particularly critical in immunity to the meningococcus; a species encountered as a commensal throughout life, which establishes a profile of responses that protects against pro-inflammatory damage following colonization. These responses may subsequently prove to be inappropriate in circumstances where the organism invades and establishes infection. The ability to modify normal DC responsiveness and trigger protective mechanisms then becomes critical, providing the potential for an alternative route towards development of a broadly-reactive mucosally-active MenB vaccine.

**Notes**

**Acknowledgments.** We thank Mark Jepson and Alan Leard for their assistance with use of the Wolfson Bioimaging Facility Light Microscopy Unit at the University of Bristol. We thank Mumtaz Virji and Natalie Griffiths for advice and assistance with microbiology experiments.

**Financial support.** This work was supported by Medical Research Council grant number G0600286.

**Potential conflicts of interest.** The authors report no conflicts of interest.
References