CD11c$^+$CD8α$^+$ dendritic cells promote protective immunity to respiratory infection with *Bordetella pertussis*

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Abstract

$CD11c^+CD8\alpha^+$ and $CD103^+$ DC have been shown to promote regulatory T cell responses and mediate tolerance in the gastrointestinal tract. These cells have also been identified in the lung, but their role in immunity to respiratory tract infection is not clear. Here we have used a murine model of infection with *Bordetella pertussis* to examine the function of DC subtypes in protective immunity in the lungs. We found a dramatic increase in the numbers of $CD11c^+CD8\alpha^+$ DC in the cervical lymph nodes (CLN) within 4 hrs of challenge with *B. pertussis* and these DC could acquire particulate antigen from the upper respiratory tract. $CD11c^+CD8\alpha^+$ DC also infiltrated the lung with a peak 7 days post *B. pertussis* challenge. The infiltrating $CD11c^+CD8\alpha^+$ DC expressed MHC, co-stimulatory and activation markers, indicative of mature DC. The $CD11c^+CD8\alpha^+$ DC in the CLN expressed IL-4 and IL-10, and lower levels of IFN-γ, but in the lungs expressed predominantly IFN-γ. Depletion of $CD8\alpha^+$ cells early in infection attenuated Th1 responses in the lungs and significantly reduced bacterial clearance. Conversely, transfer of FLT3 ligand (FL)-expanded $CD11c^+CD8\alpha^+$ DC enhanced bacterial clearance, whereas GM-CSF-expanded conventional DC had no effect. The numbers of $CD11c^+CD8\alpha^+CD103^+$ cells were also increased during the early phase of infection. Blocking CD103 function caused a significant delay in bacterial clearance and a reduction in cellular infiltration into the lungs. These findings demonstrate that $CD11c^+CD8\alpha^+$ and $CD11c^+CD103^+$DC play a protective role in mediating immunity to *B. pertussis* infection in the respiratory tract.

Introduction

B. pertussis is a Gram negative bacterium that causes the severe debilitating disease whooping cough in infants and young children (1). Despite the widespread availability of whole cell and acellular pertussis vaccines, infection still causes significant morbidity and mortality worldwide. Furthermore, adolescents and adults may act as reservoirs for the bacteria and can subsequently infect non-vaccinated infants (2, 3). The bacteria colonise the nasopharynx, trachea and bronchial tree of the lungs. Clearance of the bacteria is dependant on IFN-γ, produced locally in the lungs, initially by infiltrating NK cells (4), and then by Th1 cells (5, 6). However, infection also induces antigen-specific Th17 cells and IL-10-producing regulatory T (Treg) cells (7)(8). These Treg cells can subvert the development of adaptive immunity and delay clearance of bacteria from the lungs (8).

Immature dendritic cells (DC) are located in almost all tissues of the body, including the various compartments in the lung (9). Following activation through pathogen recognition receptors (PRR), the DC mature and migrate to the draining lymph nodes, where they present antigen to resident naïve $CD4^+$ T cells (10). DC can be subdivided based on the expression of the integrin CD11c, CD8α and CD45RA (11-16). It has been reported that $CD11c^+CD8\alpha^+$ DC can inhibit $CD4^+$ T cell activation and proliferation by causing Fas-induced cell death (13, 14). Based on these studies and others, it has been assumed that $CD11c^+CD8\alpha^+$ DC contribute to peripheral tolerance, while $CD11c^+CD8\alpha^-$ DC initiate immunity to infection. In more recent studies, CD24 and CD45RA have been used to discriminate pro-inflammatory lymphoid $CD11c^+CD8\alpha^+$ DC ($CD11c^+CD8\alpha^+CD24^+CD45RA^-$) from plasmacytoid DC (pDC; $CD11c^+CD8\alpha^+CD24^-CD45RA^+$) (11, 12). Although pDC have a pro-inflammatory role, particularly against viral infection (17, 18), these cells have been shown to possess immunoregulatory activity in mice (19) and humans (20) $CD11c^+CD8\alpha^+CD24^+CD45RA^-$ DC are pro-inflammatory and are capable of capturing antigens from other DC that migrate from

a site of infection, such as the lung (11, 12). Studies in virally infected mice have shown that this transferred antigen is subsequently presented to T cells in draining lymph nodes during the first 3 days of infection (11, 12). Unlike conventional bone marrow-derived ($CD8\alpha^-$) DC, $CD11c^+CD8\alpha^+CD24^+CD45RA^-$ DC (referred to as $CD11c^+CD8\alpha^+$ DC from here on) express the FMS-like tyrosine kinase 3 (Flt3) receptor and are not generated from bone marrow cells by GM-CSF but with Flt3-ligand (FL) *in vitro* (21).

$CD11c^+$ DC that express the $\alpha_4\beta_E$ integrin $CD103^+$, have been described in the intestine where they promote inducible regulatory T cells (iTreg) by a retinoic acid and TGF-β-dependent mechanism (22-24). Conversely, pro-inflammatory $CD103^+CD11c^{hi}CD11b^{lo}CD8\alpha^-$ DC have been detected in the mucosal lining of the lung and have been shown to play a role in a murine model of airway hypersensitivity (25, 26).

In the present study, we investigated the role of $CD11c^+CD8\alpha^+$ and $CD11c^+CD103^+$ DC in immunity to *B. pertussis* infection in mice. We used an established murine model of *B. pertussis* infection, where vaccine-induced protection correlates with efficacy in children (27). We addressed the hypothesis that $CD11c^+CD8\alpha^+$ DC may either play an immunoregulatory role by activating Treg cells or a protective role by promoting effector Th1 cells. We found significant infiltration of $CD11c^+CD8\alpha^+$ DC into the cervical lymph nodes (CLN) and then the lungs 1-7 days after infection. These DC were activated, expressed MHC, co-stimulatory molecules as well as CD103 and secreted IFN-γ, IL-4 and IL-10. Depletion of $CD8\alpha^+$ cells throughout infection and more importantly immediately before and after *B. pertussis* challenge delayed clearance of the bacteria, suggesting that these DC play a protective role very early in infection. Blocking CD103 function prior to infection also had a dramatic inhibitory effect on bacterial clearance. Finally, transfer of FL-generated $CD11c^+CD8\alpha^+$ DC prior to infection significantly enhanced bacterial clearance. Our findings

demonstrate that $CD11c^{+}CD8\alpha^{+}$ DC play a significant role in protective immunity to respiratory infection with *B. pertussis*.

Materials and Methods

Animals

BALB/c mice were purchased from Harlan UK Ltd., Oxon, UK. Animal experiments and maintenance were approved and regulated by the university ethics committee and the Irish Department of Health.

B. pertussis challenge model

Mice were challenged by aerosol exposure to live *B. pertussis*. Virulent *B. pertussis* Wellcome 28 was grown at 36^{o}C in Stainer-Scholte liquid medium. Bacteria from a 48 h culture were resuspended at a concentration of approximately 2 x 10^{10} CFU/ml in physiological saline containing 1% casein. The challenge inoculum was administered to mice over a period of 15 minutes (1.5 ml/min) with a nebuliser in a sealed container within a class 3 exhaust-protected cabinet. This was followed by a rest period of 10 min. The course of infection was followed by performing *B. pertussis* colony forming units (CFU) on lung homogenates at intervals after infection (8)

Depleting and blocking antibodies

Hybridoma cells were obtained from the ATCC and secreted IgG that was purified from hybridoma supernatants using a MAbTrap affinity chromatography kit (GE Healthcare). The optimal concentration of antibody required to deplete greater that 95% of the relevant target cells was determined by titration. Briefly, mice were injected intraperitoneally (i.p.) with increasing concentrations of depleting antibody. The spleens of these mice were subsequently investigated for the target cell 24, 48 and 72 h post injection, by flow cytometric analysis. The hybridoma cell lines, clones 2.43 and N418 were used to generate rat anti-mouse (IgG_1) CD8α and CD11c respectively. In vivo blocking of CD103 was achieved by injecting mice i.p. with anti-CD103 (clone M290). A rat anti-mouse horse radish peroxidase (HRPN) IgG_1

was used as the isotype matched control (Bioxcell) in all experiments except those using anti-CD8α. Rat anti-mouse IgG_{2b} (Sigma) was used as the isotype matched control in place of anti-CD8α (clone 2.43).

Cell isolation and purification

Infiltrating leucocytes were isolated from lungs by digesting lung tissue in PBS containing 1 mg/ml Collagenase D (Sigma) plus 0.2 μM DNAse (Sigma) for 1 hour at 37°C. T cell and DC subtypes were isolated from various tissues or from cells expanded in culture using either magnetic assisted cell sorting (MACS; Miltenyi Biotech) or using a MoFlo cell sorter (Beckman Coulter). $CD11c^+CD24^+CD45RA^-$ cells were sorted from FL-generated bone marrow DC using fluorochrome-conjugated antibodies directed against the relevant surface antigens (eBiosciences).

Flow Cytometry

Cells were stained with antibodies directed against CD3, CD4, CD8α, CD8β, CD11c, CD40, $H\text{-}2^K$, I-A/I-E, CD1d, CD24, CD45RA, GR1, B220, CD103, CD205, CD80, CD86 (eBiosciences). Labelled cells were acquired using a CyAn flow cytometer (Beckman Coulter). T cell and DC subsets were determined by gating on viable forward versus side scatter gates.

Detection of Intracellular cytokines in activated T cells and $CD11c^+$ DC

Lung cells were activated with PMA (10 ng/ml) and ionomycin (1 μg/ml) in the presence of irradiated (30 Gy from a ^{137}Cs source) spleen cells for 4 h, and then incubated overnight at 37°C in the presence of Brefeldin A (BFA; Sigma). Alternatively $CD4^+$ T cells were activated with antigen *in vivo* by injecting mice with $4x10^9$ heat-killed *B. pertussis* i.p. 4 h prior to

removal of lungs. Lung mononuclear cells were incubated with BFA overnight without additional stimulus. Cell were permeabilised and stained with antibodies directed against IL-4, IL-10, IL-17, and IFN-γ. Intracellular cytokine expression in DC was determined directly, by staining isolated cells with antibodies directed against relevant antigens, without re-stimulation or incubation with BFA.

H&E staining of lung tissue sections

Lungs were removed from the animals and fixed overnight in 10% neutral buffered formalin. Tissues were then processed overnight to paraffin wax and embedded. Blocks were sectioned at 4 μm and were stained with H&E by a Cot-20 automated linear stainer (Midite).

In vitro activation of $CD4^+$ helper T cells from B. pertussis-infected mice with DC

$CD11c^+CD8\alpha^+$ DC were sorted from lung homogenates on day 7 after infection using a MoFlo cells sorter. $CD4^+$ T cells purified from spleens of infected mice using CD4 T cell isolation kits (Miltenyi Biotech). 0.2 x 10^5 DC and 1 x 10^5 T cells were cultured in RPMI medium supplemented with L-glutamine and 10% FCS in wells of a 96 well plate and activated with 2 μg/ml immobilised anti-CD3. After 48 h, supernatants were removed and analysed for IL-10, IL-12p70, IFN-γ and IL-4 by ELISA (R&D systems). Proliferation was assessed 72 h post activation by tritiated thymidine incorporation.

Generation of $CD11c^+CD24^+CD45RA^-$ cells with FL *in vitro*

Bone marrow cells were isolated from the femurs of BALB/c mice, cultured at a concentration of 2 x 10^6 cells per well in Stemline dendritic cell maturation medium (Sigma) supplemented with 0.3 g/L L-glutamine and 300 ng/ml recombinant FL (ImmunoTools). Cells were incubated in humidified air for 8 days at 37°C and in 10% CO_2. Additional FL

(300 ng/ml) was added on days 3 and 6. These cells were subsequently washed and stained with antibodies against CD24, CD45RA and CD11c. $CD11c^{+}CD24^{+}CD45RA^{-}$ cells (FL-DC) were purified using a MoFlo cell sorter and counted. 0.5 x 10^{5} $CD11c^{+}CD24^{+}CD45RA^{-}$ cells were injected i.p. into mice 30 min after infection.

Uptake of Green fluorescent latex bead (FLB) particles by DC

Plain yellow-green fluorescent 0.5μm latex particles (Polysciences) were diluted 1:25 in PBS for a final administration of approximately 3.64×10^{8} particles per mouse. 20 μl of this solution was administered intra-nasally (i.n.) 15 min prior to infection. Control mice received latex particles in the absence of infection.

Statistical Analysis

Results, expressed as mean ± SEM, were compared using the Student's t test or by ANOVA. *P* values <0.05 were considered statistically significant.

Results

$CD11c^+CD8\alpha^+$ DC infiltrate the lungs and draining lymph nodes during acute phase of infection with B. pertussis

We investigated the infiltration and function of $CD11c^+CD8\alpha^+$ lymphoid DC in the lungs and draining lymph nodes of mice through the course of infection with *B. pertussis*. Respiratory challenge of naive BALB/c mice by exposure to an aerosol of live *B. pertussis* resulted in a reproducible non-lethal infection with peak bacterial counts on day 14 and approached complete clearance by 28 days (Fig 1A). The relative percentage of $CD11c^+CD8\alpha^+$ DC was determined by gating on viable lymphocytes from forward versus side scatter plots in conjunction with the appropriate isotype and compensation controls. The total number of cells expressing various surface antigens was calculated based on the total number of viable cells and the percentage of $CD11c^+CD8\alpha^+$ cells within the viable lymphocyte gate. The numbers of $CD11c^+$ and $CD11c^+CD8\alpha^+$ DC were low in the CLN, thoracic lymph nodes (TLN) and lungs of non-infected control mice (Fig. 1B-D). However, during *B. pertussis* infection, we consistently found an early influx of $CD11c^+$ and $CD11c^+CD8\alpha^+$ DC into the CLN, and a later influx into the lungs (Fig. 1B-D). The numbers of both DC subsets in the CLN increased dramatically between 4 and 24 h of aerosol challenge with live *B. pertussis* and steadily diminished thereafter to baseline levels (Fig. 1B, C). Conversely, the number of $CD11c^+$ and $CD11c^+CD8\alpha^+$ DC increased in the lung from day 1 and peaked on days 5-7 of infection. The number of DC subsets in the draining lymph nodes and lungs declined to that in control mice after 14-21 days (Fig. 1B,C). There was a small, but not statistically significant, increase in the frequency of $CD11c^+CD8\alpha^+$ DC in the TLN 3 days after *B. pertussis* aerosol challenge (Fig. 1C). However the number had returned to baseline values by day 5 (Fig 1C) and remained there when examined 7, 14 and 21 days post challenge (data not

shown). Our findings suggest that following *B. pertussis* aerosol challenge, DC are recruited to the CLN and then may migrate to the lungs.

$CD11c^+CD8\alpha^+$ DC in the CLN acquire particulate antigen from the respiratory tract within 4 hours of respiratory challenge with B. pertussis

We have shown that $CD11c^+CD8\alpha^+$ DC with a mature phenotype are present in the CLN of mice within 24 h of *B. pertussis* challenge. However, it is unclear whether *$CD11c^+CD8\alpha^+$ D*C migrate from the lung or reside in the CLN and acquire antigen from other migrating cells types. A recent report suggested that $CD11c^+CD8\alpha^+$ DC are resident in the draining lymph nodes and rarely migrate (28). Therefore, we hypothesised that these cells acquire particulate antigen from dying APC that migrate from the lung and in turn present antigen via MHC to T cells within the lymph node. In order to test this hypothesis, green fluorescent latent beads FLB were administered i.n. to mice 15 min prior to infection with *B. pertussis* and the number of FLB^+ DC were determined in the CLN and lung at different time points after infection. FLB were found in the total leucocyte population and in $CD11c^+CD8\alpha^+$ DC isolated from the CLN 4 h after infection (Fig. 2A). On the other hand, very few total FLB^+ leucocytes or $FLB^+CD11c^+CD8\alpha^+$ DC were detected in the CLN or lungs of non-infected mice up to day 3 post infection (Fig. 2B and data not shown). Furthermore, very few FLB^+ cells were detected in the lungs from either infected or non-infected control mice up to day 7 post challenge (data not shown). These findings suggest that $CD11c^+CD8\alpha^+$ DC in the CLN can acquire particulate antigen from the upper respiratory tract within hours of infection. It remains unclear however whether or not the $CD11c^+CD8\alpha^+$ DC activated in the CLN are the same as those observed in the lung some days later. Based on this data it would seem that they are not the same cell.

$CD11c^+CD8\alpha^+$ DC cells from the CLN and lungs of infected mice are mature and secrete pro-inflammatory cytokines

We next examined the activation status of $CD11c^+CD8\alpha^+$ DC in the CLN and lungs. Infiltrating $CD11c^+CD8\alpha^+$ DC expressed surface markers indicative of maturation, but at different stages of infection in the CLN and lungs. Expression of MHC class I and CD40 was enhanced on $CD11c^+CD8\alpha^+$ DC in the CLN, with peak expression 1 day after challenge (Fig. 3A, B). MHC class II expression was also enhanced with peak expression on day 3. A similar pattern was observed on $CD11c^+CD8\alpha^+$ DC in the lung, except that peak expression of these surface antigens was delayed by at least 24 hours when compared with the CLN (Fig. 3A, B). We also investigated whether $CD11c^+CD8\alpha^+$ DC expressed surface CD205, a marker of antigen uptake, during acute infection with *B. pertussis*. CD205 expression was significantly enhanced in the CLN after 24 hours and lungs 36-48 hours after *B. pertussis* challenge and gradually declined during the first 7 days of infection (Fig. 3A, B). The number of $CD11c^+CD8\alpha^+$ cells expressing either CD80 or CD86 DC peaked by day 3 and 5 in the CLN and lungs respectively in mice post challenge with *B. pertussis* (Fig. 3A). Finally, the majority of $CD11c^+CD8\alpha^+$ DC from CLN and lungs early in infection expressed CD24 (data not shown).

$CD11c^+$ DC have been shown to secrete IFN-γ, IL-12p70, IL-4 and IL-10 in a variety of murine models of infection and inflammation (29-34). Therefore, we performed intracellular cytokine staining on $CD11c^+CD8\alpha^+$ DC during the acute stage of infection with *B. pertussis*. Cells isolated from the CLN or lungs of infected mice were permeabilised and stained directly with anti-cytokine antibodies, without re-activation or incubation with Brefeldin A *in vitro*. A significant number of $CD11c^+CD8\alpha^+$ in the CLN secreted IL-4 and IL-10 and this peaked 4 days after infection. A smaller number of $CD11c^+CD8\alpha^+$ DC in the CLN of *B. pertussis* infected mice secreted IFN-γ (Fig. 4). In contrast, a high frequency of

IFN-γ-secreting $CD11c^+CD8\alpha^+$ DC were detected in the lungs of infected mice, especially 3-5 days after *B. pertussis* challenge (Fig. 4). IL-4 and IL-10 secreting $CD11c^+CD8\alpha^+$ DC were also detected in the lungs, but at lower frequency than the IFN-γ-secreting $CD11c^+CD8\alpha^+$ DC (Fig. 4). Although a number of $CD11c^+CD8\alpha^+$ DC expressed both IL-4 and IFN-γ or IL-10 and IFN-γ in the CLN, very few co-producers were detected at any stage during acute infection in the lung (Fig. 4). The percentage of $CD11c^+CD8\alpha^+IFN\text{-}\gamma^+$ DC was lower than $CD11c^+CD8\alpha^+IL\text{-}4^+$ or $CD11c^+CD8\alpha^+$ $IL\text{-}10^+$ DC in the CLN on day 4 of infection when compared with non-infected control mice (Fig. 4B, C). In contrast, the percentage of $CD11c^+CD8\alpha^+IFN\gamma^+$ DC was increased in the lungs of infected animals on day 5 (Fig. 4D). These findings demonstrate that activated $CD11c^+CD8\alpha^+$ DC found in the CLN soon after respiratory challenge with *B. pertussis* predominantly express IL-4 and IL-10 with a lower frequency expressing IFN-γ, whereas the $CD11c^+CD8\alpha^+$ DC infiltrating the lungs a few days later express predominantly IFN-γ.

$CD11c^+CD8\alpha^+$ DC from infected mice can induce proliferation and pro-inflammatory cytokine secretion by $CD4^+$ T cells in vitro

We next investigated whether $CD11c^+CD8\alpha^+$ DC were as effective as total $CD11c^+$ DC at stimulating $CD4^+$ T cells and if the activated T cells had a similar cytokine profile. Total $CD11c^+$ DC or $CD11c^+CD8\alpha^+$ DC were sorted from the lungs of mice, at the peak of infiltration 7 days post challenge with *B. pertussis* (Fig. 5A) and co-cultured *in vitro* with $CD4^+$ T cells purified from the spleens of infected mice. $CD11c^+CD8\alpha^+$ DC were as effective as total $CD11c^+$ DC in inducing proliferation of $CD4^+$ T cells in response to anti-CD3 (Fig. 5B). IFN-γ (Fig. 5C) and IL-4 (Fig. 5D) were also detected in supernatants of $CD4^+$ T cells stimulated with $CD11c^+CD8\alpha^+$ DC as well as $CD11c^+$ DC, although the concentrations were higher with the latter (Fig. 5C). IL-10 and IL-12p70 production were un-detectable in

supernatants from $CD4^+$ T cells stimulated with either population of DC (data not shown). The results show that $CD11c^+CD8\alpha^+$ DC from the lungs of mice during the acute stage of infection with *B. pertussis* can activate $CD4^+$ T cells *in vitro*.

Depletion of $CD8\alpha^+$ cells impairs bacterial clearance

Having shown that functionally active, mature $CD11c^+CD8\alpha^+$ DC infiltrate into the lungs during acute infection with *B. pertussis*, we examined the hypothesis that they may play a positive role in anti-bacterial immunity and thereby function in bacterial clearance. In order to examine the function of $CD8\alpha^+CD11c^+$ DC rather than total $CD11c^+$ DC, we depleted $CD8\alpha^+$ cells *in vivo* using an anti-CD8α depleting antibody. Although this approach will also deplete $CD8^+$ T cells, unlike other approaches (35) it will not deplete other DC subsets. $CD8\alpha^+$ cells were depleted from mice infected with *B. pertussis* by injecting anti-CD8α antibodies i.p. on days -1, +1, +3, +6, +13, +19 post *B. pertussis* challenge. Depletion of CD8α was confirmed by flow cytometric analysis on spleen cells (Fig.6A). Depleting $CD8\alpha^+$ cells throughout infection had a marginal impact on the numbers of total $CD11c^+$ cells in the lung (Fig. 6B), however, $CD11c^+CD8\alpha^+$ DC were almost completely absent from the lungs of infected mice treated with anti-CD8α antibody (Fig. 6C).

Depletion of $CD8\alpha^+$ cells from mice throughout infection with *B. pertussis* delayed bacterial clearance; the CFU counts were significantly higher ($p<0.01$) in depleted animals when compared with *B. pertussis*-infected control mice 21 days post challenge (Fig. 6 D). This was a consistent finding in 6 experiments. Furthermore, deaths were observed amongst the group of mice depleted of $CD8\alpha^+$ cells, a feature previously observed in mice lacking functional IFN-γ (6). We next investigated the impact of depleting $CD8\alpha^+$ cells (throughout infection) on $CD4^+$ T cell cytokine production. Cells were isolated from the lungs of infected animals 30 days post challenge and intracellular cytokine staining was

performed. IFN-γ-secreting and IL-10-secreting $CD4^+$ T cells were detected in the lungs of mice infected with *B. pertussis* (Fig 6E). In contrast, IL-17-producing T cells were almost undetectable. The frequency of $CD4^+IFN\text{-}\gamma^+$ T cells infiltrating the lung 30 days post infection was reduced in CD8α-depleted compared with non-infected control and non-depleted *B. pertussis*-infected control mice (Fig. 6E). Conversely, the percentages of $CD4^+IL\text{-}10^+$ T cells and $CD4^+IL\text{-}17^+$ T cells were enhanced in CD8α-depleted mice (Fig. 6E). These findings are consistent with the data in Fig 5 showing that CD8α DC could activate IFN-γ producing $CD4^+$ T cells.

In order to determine the role of $CD8\alpha^+$ cells at different stages of infection, infected mice were injected with anti-CD8α depleting antibody either on day -1 and +1, from day -1 through to +19 or from +6 through to +19. Depleting $CD8^+$ cells either early in infection or throughout the course of infection resulted in a significant enhancement of bacterial burden in the lungs ($p < 0.05$), when compared with infected control mice (Fig. 7A). In addition, the number of cells in the perivascular spaces of lung tissue (isolated 30 days post infection) was greater in mice depleted of $CD8\alpha^+$ cells either during the early phase or throughout infection when compared with *B. pertussis* infected un-depleted control animals (Fig. 7B).

Since *B. pertussis* causes a predominant extracellular infection, it was assumed that $CD8^+$ T cells would not play a protective role in immunity to *B. pertussis*, in particular during the acute phase of infection. Indeed, we have previously demonstrated that transfer of $CD8^+$ T cells exacerbated infection with *B. pertussis* (5). Nevertheless we examined the possibility that the enhanced infection seen in CD8-depleted mice resulted form depletion of $CD8^+$ T cells. We first examined the infiltration of $CD8^+$ T cells and found that they do infiltrate into the lungs of *B. Pertussis*-infected mice, especially late in infection (Fig. 7C). Depletion of $CD8\alpha^+$ cells from day 6 after *B. pertussis* challenge significantly ($p< 0.05$) enhanced bacterial clearance when compared with non-depleted infected control mice (Fig. 7D). This

observation points to a possible regulatory role for $CD8^+$ T cells in immunity to *B. pertussis*. In contrast, the enhancement of infection observed following depletion of $CD8^+$ cells early in infection together with the early appearance of $CD8\alpha^+$ DC suggest a protective role for this DC population in immunity to *B. pertussis.*

Transfer of in vitro-expanded FL-DC into infected mice enhances cellular infiltrate and promotes bacterial clearance from the lung

The results of the depletion experiments suggested that $CD11c^+CD8\alpha^+$ DC contributed to anti-bacterial immunity in the lungs; we sought to confirm these findings using a cell transfer approach. We used FL to expand $CD11c^+CD24^+CD45RA^-$ (FL-DC) from freshly isolated bone marrow cells and removed $CD11c^+CD24^-CD45RA^+$ pDC by cell sorting (36). CD8α is not expressed on these cells *in vitro*; however, CD8α expression is up regulated when they are transferred *in vivo* (37). The $CD11c^+CD24^+CD45RA^-$ (FL-DC), which were routinely 95% pure (Fig. 8A), were transferred 30 minutes after *B. pertussis* challenge. Transfer of FL-DC significantly enhanced bacterial clearance; when compared with *B. pertussis* infected controls. Mice that received the DC transfer had significantly lower ($p<0.05$) CFU counts, particularly on day 14 post challenge (Fig. 8B). In order to exclude the possibility that transfer of any DC subset could enhance bacterial clearance in *B. pertussis*-infected animals, experiments were performed where mice received either DC expanded in culture with GM-CSF and IL-4 (GM-DC) or FL-DC 30 min after infection. Only FL-DC transfer significantly accelerated bacterial clearance ($p<0.05$) in infected animals (Fig. 8C).

Transfer of FL-DC was associated with an enhancement, in the number of $CD4^+$ T cells and B cells but not neutrophils infiltrating the lungs (Fig. 8D). Although not statistically significant, this was a consistent finding over 5 separate experiments (data not shown).

Finally, the numbers of $CD11c^+$ or $CD11c^+CD8\alpha^+$ DC in the lungs of infected mice were not significantly altered by transfer of FL-DC, when examined 7 days after challenge (Fig. 8E).

Blocking CD103 function in vivo impairs bacterial clearance

A number of studies have reported that DC, particularly in the gut and lung express the $\alpha_4\beta_E$ integrin, CD103, whose ligand, E-cadherin, is expressed by epithelial cells on mucosal surfaces (23, 25). Therefore, we examined expression of CD103 on DC in the lungs and CLN of *B. pertussis*-infected mice. We found a significant increase in the percentage and number of $CD11c^+CD8\alpha^+CD103^+$ DC in the CLN on day 4 post infection when compared with non-infected control mice (Fig. 9A, B). In contrast, significant numbers of $CD11c^+CD8\alpha^+CD103^+$ DC were detected in lung by day 6 post challenge (Fig. 7A,B). It is possible that $CD11c^+CD8\alpha^+$ DC from the CLN require surface CD103 to track into the lung by interacting with E-cadherin on epithelial cells in the mucosal surface. We next investigated the possible role of CD103 in protective immunity to *B. pertussis* infection. Mice were injected once with an anti-CD103 blocking antibody 24 h prior to infection, It was not possible to specifically eliminate $CD11c^+CD8\alpha^+CD103^+$ cells, as only 40% of the $CD103^+$ cells in either CLN or lung, during the acute phase of infection, express CD8α (data not shown). Nevertheless, blocking CD103 function with a single injection of anti-CD103 antibody, had a dramatic impact on bacterial clearance, with a significant ($p<0.01$), enhancement in CFU counts 21 days post *B. pertussis* challenge (Fig. 9C). Inhibition of CD103 constrained cellular infiltration into the lungs 21 days after challenge (Fig. 9D). These findings suggest that $CD11c^+CD103^+$ DC infiltrate the CLN and then the lungs in the early stages of infection with *B. pertussis* where they promote cellular immune responses that mediate clearance of the bacteria from the respiratory tract.

Discussion

$CD11c^+CD8\alpha^+$ DC of lymphoid origin have been considered as primarily immunoregulatory (34, 38-40), however, it has been suggested that $CD11c^+CD8\alpha^+$ may also have pro-inflammatory functions (29, 30, 33, 41-44). In this study we have demonstrated that $CD11c^+CD8\alpha^+$ DC infiltrate the CLN and then the lungs of mice early after infection with *B. pertussis*, express a number of maturation markers and secrete IFN-γ as well as IL-10 and IL-4. Depletion of these cells early in infection reduced the frequency of IFN-γ-secreting $CD4^+$ cells infiltrating the lung and delayed bacterial clearance. Furthermore, transfer of FL-DC enhanced bacterial clearance, demonstrating that $CD11c^+CD8\alpha^+$ DC play a protective role in immunity to infection with *B. pertussis*.

It is widely accepted that $CD11c^+CD8\alpha^+$ cells are the principle DC subset involved with cross presentation of cell-associated antigens by MHC class II (41, 45, 46). We found that $CD11c^+CD8\alpha^+$ DC that infiltrate the draining lymph nodes very soon after infection with *B. pertussis* had enhanced expression of a number of activation markers, including CD40, CD80, CD86, MHC class I and II. This profile changes in $CD11c^+CD8\alpha^+$ DC that appear in the lungs a few days later, with higher expression of MHC class II and lower expression of CD40. Furthermore, we found that $CD11c^+CD8\alpha^+$ DC in the CLN acquire intra-nasally delivered fluorescent particles within hours of infection. Since these cells are very efficient at acquiring antigen from apoptotic and necrotic cells (47), it is possible that they are capable of acquiring antigen from newly arrived apoptotic antigen-presenting cells in the CLN of mice infected with *B. pertussis* and thereby activating MHC class II restricted $CD4^+$ T cells, which play a critical role in protection against *B. pertussis*.

Interestingly, expression of CD205, a marker of endocytic activity and antigen internalisation (48), was also enhanced on $CD11c^+CD8\alpha^+$ DC early in infection and this inversely correlated with CD103 in particular in the lung. It has been suggested that CD205

on the surface of DC might interfere with CD103 integrin binding to its ligand E-cadherin on endothelial cells (49). Therefore, it is possible that during the initial phase of antigen up-take and processing, the DC is prevented from migrating from the CLN to the lung, but on maturation, CD205 expression diminishes and CD103 increases, which would permit the DC to migrate to the mucosal surface. However, we have yet to elucidate whether *C*D11c$^+$CD8α$^+$ DC observed in the draining lymph nodes are the same cells seen in the lung some days later.

Although the precise mechanism by which CD8α$^+$ DCs mediate protection against *B. pertussis* infection is not clear, the findings suggest that they promote a protective Th1 response in the CLN and lungs, which is mediated by innate IFN-γ production. CD11c$^+$CD8α$^+$ DC from the CLN of *B. pertussis* infected mice expressed significant IL-4 and IL-10 and a lower frequency expressed IFN-γ. This is consistent with the regulatory phenotype attributed to these cells (34, 38). However, we found that CD11c$^+$CD8α$^+$ DC that infiltrated the lungs a little later in the bacterial infection produced predominantly IFN-γ, with a lower frequency expressing IL-4 and IL-10. CD11c$^+$CD8α$^+$IFN-γ$^+$ DC primed with bacterial antigen might become the dominant subset in the lung as a result of activation through TLR ligands or other bacterial derived PAMPs or through interaction with other innate cells and CD4$^+$ T cells within the lung. Interestingly Th1 responses were reduced and IL-10-producing T cells enhanced in mice depleted of CD8α$^+$ cells. Thus CD11c$^+$CD8α$^+$IFN-γ$^+$ DC may help to promote Th1 responses, which help to clear the infection. The simultaneous presence of IL-10-secreting DC in the lungs and CLN is consistent with the co-induction of Th1 and Tr1-type cells, and the role of the latter cells in suppressing effector responses early in infection and in controlling immunopathology in the lungs (8, 50).

Cell transfer and depletion experiments demonstrated that CD11c$^+$CD8α$^+$ DC made a positive contribution to protective immunity in the respiratory tract. Depleting CD8α$^+$ cells during the first 7 days of infection with *B. pertussis* delayed bacterial clearance, implying that

$CD11c^+CD8\alpha^+$ DC play a significant role in initiating protective immunity to infection with this organism. It has previously been suggested that $CD11c^+CD8\alpha^+$ DC are required for the generation of protective virus-specific CTL responses in mice respiratory infected with influenza virus (32). However, that conclusion was based on depletion of total $CD11c^+$ cells, which included myeloid and lymphoid DC (32). In contrast to the results described here, Leef et al have found that administering the same $CD8\alpha^+$ depleting antibody to that used in our study, had little or no effect on the course of bacterial colonisation with *B. pertussis* in mice (51). However, in that study, mice were immunized with formalin-fixed bacteria or micro-encapsulated pertussis antigens, prior to *B. pertussis* challenge (51). Therefore, $CD11c^+CD8\alpha^+$ DC might have a significant role in primary infection in the lung, but perhaps a lesser role during secondary immune responses. We also demonstrated that transfer of $CD11c^+CD24^+CD45RA^-$ DC expanded in vitro with FL, and which acquire CD8α when transferred in vivo (37), consistently caused a small but significant enhancement in bacterial clearance in infected mice. This was not observed with DC expanded with GM-CSF and IL-4. It has been shown that repeated injections of mice with high concentrations of recombinant FL greatly expands $CD11c^+CD8\alpha^+$ in vivo (16). Indeed FL has been used in phase I and II clinical trials as an adjuvant in a vaccine against Hepatitis B infection (44, 52-54) and has been used to treat a number of different cancers in mice and humans including metastatic lung cancer (55, 56). Our results indicate that FL represents an exciting therapeutic option against lung infections. However, it has been shown recently that transfer of $CD11c^+CD8\alpha^+$ DC enhanced HSV type I latency in mice (39). Since FL expands both $CD11c^+CD8\alpha^+CD24^+CD45RA^-$ DC and pDC it is possible that the latter subset is responsible for immunoregulation and enhanced latency, whereas the former may be pro-inflammatory and protect against infection.

We also found evidence that CD103 has a positive role in driving cellular immunity against *B. pertussis* infection. We found significant infiltration of $CD11c^{+}CD8\alpha^{+}CD103^{+}$ DC into the CLN and lungs 4-6 days after reparatory challenge with *B. pertussis*. Furthermore, injection of a blocking antibody to CD103 prior to infection caused a significant delay in clearance and in infiltrating lymphocytes in the lungs. Since CD103 is also expressed on other cell types, we cannot exclude their role in protection, but our data do suggest that $CD11c^{+}CD8\alpha^{+}CD103^{+}$ DC may have a protective function in the lungs. This conclusion is consistent with recent studies describing pro-inflammatory $CD103^{+}CD11c^{hi}CD11b^{lo}$ DC that reside in the lung mucosa and vascular wall (25, 26). In short, DC that express CD103 can have pro-inflammatory function. These DC have been shown to migrate across the airway epithelia for antigen transport and presentation and contribute to the pathogenesis of airway hypersensitivity in mice (25). Furthermore, they express CCR7, which allows them to migrate to the bronchial nodes during viral infection (26). Therefore, it is possible that blocking CD103 function during *B. pertussis* infection inhibited $CD11c^{+}CD8\alpha^{-}CD103^{+}$ in the lung and in the CLN, causing a delay in antigen transfer from the lung to the draining lymph nodes. A protective pro-inflammatory role for $CD11c^{+}CD103^{+}$ DC in the lungs is at variance with the proposed regulatory function of these cells in the gut, where they have been shown to generate inducible Treg cells from effector $CD4^{+}$ T cells, by a retinoic acid and TGF-β-dependent mechanism (22-24). However, pro-inflammatory $CD11c^{+}CD103^{+}$ DC have also been described in murine models of airway hypersensitivity and viral infection (25, 26). Therefore it is possible that CD103 might not be a marker of pro-inflammatory or regulatory DC per se but an integrin, which is involved with DC homing between mucosal surfaces and draining lymph nodes.

In conclusion, we hypothesise that lymph node resident $CD11c^{+}CD8\alpha^{+}$ DC can acquire *B. pertussis* antigen from dying macrophages or other DC subtypes. These cells could

traffic to the site of infection, perhaps with the aid of surface CD103, within the lung where they become polarised to predominantly secrete IFN-γ and subsequently promote a Th1 response. Thus, $CD11c^{+}CD8\alpha^{+}$ cells represent a DC subset that is crucial for establishing immunity against *B. pertussis* infection of the respiratory tract.

Footnotes

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[3]Abbreviations used in this paper: CLN, cervical lymph node; DC, dendritic cell; pDC, plasmacytoid DC; FL, Flt3-ligand; CFU, colony forming units; FLB, green fluorescent latex bead; MLN, mesenteric lymph nodes; i.n, intranasally

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Figure Legends

FIGURE 1. Recruitment of $CD11c^+CD8\alpha^+$ DC to the lungs and draining lymph nodes soon after respiratory challenge with *B. pertussis*. Mice were infected by exposure to an aerosol of live virulent *B. pertussis. A*, The course of infection was followed by performing CFU counts on lung homogenates at intervals after *B. pertussis* challenge infected mice. *B-D*, The CLN, TLN and lungs were removed and digested with Collagenase D at various time points post challenge. Cells were counted and stained for CD11c and CD8α. The relative percentage of $CD11c^+CD8\alpha^+$ DC was determined by gating on viable lymphocytes from forward by side scatter plots in conjunction with the appropriate isotype and compensation controls. The total number of $CD11c^+$ (*B*) or $CD11c^+CD8\alpha^+$ (*C, D*) was calculated from the total number of viable cells and the percentage of $CD11c^+$ or $CD11c^+$ $CD8\alpha^+$ cells within the viable lymphocyte gate. *E*, representative FACS profiles for $CD11c^+CD8\alpha^+$ cells in either CLN or lung tissue from naive control mice or *B. pertussis* infected mice 1 and 4 days post challenge. These results are representative of 4 separate experiments. * $p<0.05$, ** $p<0.01$, versus non-infected control mice (day 0).

FIGURE 2. Intranasally-delivered green fluorescent latex beads (FLB) are detectable in $CD11c^+CD8\alpha^+$ DC from the CLN of mice 4 h after *B. pertussis* challenge. Green FLB were administered i.n. to mice, which were infected with *B. pertussis* 15 min later. CLN from non-infected and *B. pertussis* infected mice were examined after 4 h for the presence of total FLB^+ cells within the viable leucocyte gate and $FLB^+CD11c^+CD8\alpha^+$ cells. *A*, Histogram gated on viable leucocyte gate, representing $FLB^+CD11c^+CD8\alpha^+$ DC from the CLN, 4 h after infection (white histogram, black line). FLB^+ positive control (grey histogram) represents

cells isolated from the CLN of non-infected animals and incubated for 15 min at room temperature with FLB. FLB only (dashed line) represents cells from the CLN of animals that received FLB in the absence of *B. pertussis* infection. CLN cells from the CLN of *B. pertussis*-infected animals that did not receive any $FLB^+CD11c^+CD8\alpha^+$ (black histogram). *B*, The total number of FLB^+ cells or $FLB^+CD11c^+CD8\alpha^+$ DC in CLN or lungs of mice 4 h post challenge with *B. pertussis*. These results are representative of 2 separate experiments. ** $p<0.01$, versus non-infected control mice.

FIGURE 3. $CD11c^+CD8\alpha^+$ DC cells from the CLN and lungs of *B. pertussis* infected mice express a number of activation and maturation markers. Mice were infected by exposure to an aerosol of live virulent *B. pertussis.* The CLN and lungs were removed and digested with Collagenase D at various time points post infection. Cells were counted and stained for CD11c and CD8α with CD40, MHC class I, MHC class II, CD205, CD80 and CD86. *A*, The total number of cells expressing various surface antigens was calculated based on the total number of viable cells and the percentage of $CD11c^+CD8\alpha^+$ cells within the viable lymphocyte gate. *B*, Representative dot plots showing peak expression of co-stimulatory molecules and activation markers within the $CD11c^+CD8\alpha^+$ gate. Control dot plots represent cells isolated from the CLN of non-infected control mice. CLN and lung dot plots represent peak expression of MHC-I and CD40 on days 1 and 2, CD205 on days 2 and 3, MHC-II on days 3 and 5, and CD80/CD86 expression 3 and 5 days respectively after challenge with *B. pertussis* (*B*). Results are representative of 4 separate experiments. * $p<0.05$, ** $p<0.01$, versus non-infected control mice.

FIGURE 4. $CD11c^+CD8\alpha^+$ DC from the CLN and lungs of infected mice express IFN-γ, IL-10 and IL-4. Mice were infected by exposure to an aerosol of live virulent *B. pertussis,* and

the CLN and lungs were removed and digested with Collagenase D at various time points post challenge. Cells were permeabilised and stained directly with anti-IL-4, IL-10 and IFN-γ antibodies without re-stimulation or incubation with BFA. *A*, The total number of cells expressing various cytokines was calculated based on the total number of viable cells and the percentage of $CD11c^+CD8\alpha^+$ cells within the viable lymphocyte gate. Representative dot plots showing cells from the CLN of non-infected control mice (*B*), CLN (*C*) and lungs (*D*) of *B. pertussis* infected mice on day 4 post challenge. Results are representative of 4 separate experiments. * $p<0.05$, ** $p<0.01$, versus non-infected control mice.

FIGURE 5. $CD11c^+CD8\alpha^+$ cells from the lungs of *B. pertussis* infected mice can stimulate $CD4^+$ T cells *in vitro*. *A*, Infiltration of $CD11c^+$ or $CD11c^+CD8\alpha^+$ DC into lungs at intervals after *B. pertussis* challenge. *B*, $CD11c^+$ or $CD11c^+CD8\alpha^+$ DC were sorted from lung homogenates of mice on day 7 of respiratory infection with *B. pertussis*. Each cell subset was activated with 2 μg/ml of immobilised anti-CD3 in the absence or presence of conventional $CD4^+$ T cells (purified from the spleens of infected animals). Proliferation was determined by ^{3}H-thymidine incorporation 72 h later * $p<0.05$, ** $p<0.01$, versus non-infected control mice. The concentrations of IFN-γ (*C*) and IL-4 (*D*) in the supernatants were quantified by ELISA 48 h after activation. These results are representative of 2 separate experiments. Results were compared using a student's t-test. * $p<0.05$, ** $p<0.01$, versus DC or T cell alone.

FIGURE 6. Depleting $CD8\alpha^+$ cells delays bacterial clearance in infected mice. Mice were infected by exposure to an aerosol of live virulent *B. pertussis*. Mice received either 200 μg/mouse anti-CD8α depleting antibody (aCD8α) or irrelevant IgG_{2b} isotype control antibody (200 μg/mouse) i.p. 24 h prior to infection and on days 1, 3, 7, 14 and 19 thereafter. *A*, FACS

analysis on spleen cells isolated from CD8α-depleted mice, 24 h after injection showing absence of $CD8\alpha^+$ cells. The number of $CD11c^+$ (*B*) and $CD11c^+CD8\alpha^+$ (*C*) DC infiltrating the lung in *B. pertussis* infected mice control mice and mice treated with anti-CD8α 24 h prior to challenge with *B. pertussis* and on days 1, 3, 7, 14 and 19 (-1 to +19). *D*, The course of infection in *B. pertussis*-infected control mice and in CD8α-depleted infected animals was followed by perfuming CFU counts on lung homogenates at intervals after the initial challenge. One out of five animals died from the CD8α-depleted group post challenge with *B. pertussis* (†).** $p<0.01$, versus non-depleted *B. pertussis* infected mice *E*, Representative dot plots, gated on viable $CD3^+CD4^+$ T cells, showing intracellular cytokine expression in cells isolated from the lungs of mice from control and experimental groups on day 30 after *B. pertussis* challenge. These results are representative of 4 separate experiments.

FIGURE 7. Depleting $CD8\alpha^+$ cells during the first 48 h after *B. pertussis* challenge attenuates bacterial clearance and delays cellular infiltration. Mice were infected by exposure to an aerosol of live virulent *B. pertussis.* Mice received either 200 μg/mouse anti-CD8α depleting antibody (aCD8α) or irrelevant IgG_{2b} isotype control antibody (200 μg/mouse) i.p., on days -1 and +1, on days -1, +1, 3, 7, 14 and 19 (-1 to +19). *A*, bacterial colonisation in *B. pertussis*-infected control mice and in CD8α-depleted infected animals was assessed by performing CFU counts on lung homogenates on day 21 after *B. pertussis* challenge. *B*, H&E staining of paraffin-embedded lung tissue sections (x40) from *B. pertussis*-infected control mice or infected mice that were treated on days -1 and +1 or on days -1, +1, 3, 7, 14 and 19 (-1 to +19) with anti-$CD8\alpha^+$ depleting antibody. Arrows show greater numbers of immune cells infiltrate the perivascular spaces in anti-CD8α-depleted compared with control *B. pertussis* infected mice. *C*, The number $CD3^+CD8^+$ T cells infiltrating the lungs of mice up to 28 days post *B. pertussis* challenge. * $p<0.05$, ** $p<0.01$, versus non-infected control mice. *D.* Mice

were treated with anti-CD8α or irrelevant IgG_{2b} isotype control antibody (200 μg/mouse) i.p. on days +6, 14 and 19 (+6 to +19). Bacterial colonisation was assessed by performing CFU counts on lung homogenates on day 21 after *B. pertussis* challenge. * $p<0.05$, versus control non-depleted *B. pertussis* infected mice. Results are representative of 4 separate experiments.

FIGURE 8. Transfer of *in vitro*-expanded FL-DC prior to *B. pertussis* challenge enhances bacterial clearance. $CD11c^+CD24^+CD45RA^-$ DC (FL-DC) were generated *in vitro* from freshly isolated bone marrow cells cultured for 8 days in DC medium supplemented with 300 ng/ml of FL. *A*, Representative dot plots showing $CD11c^+CD24^+CD45RA^-$ DC, gated on viable $CD11c^+$ cells, which were sorted prior to transfer *in vivo*. *B*, The course of infection in *B. pertussis*-infected control mice and in infected animals that received 0.5×10^5 FL-DC (30 min prior to challenge), was followed by performing CFU counts on lung homogenates at fixed time points after the initial challenge. * $p<0.05$ versus control *B. pertussis* infected mice. *C*, The bacterial load in *B. pertussis*-infected control mice and in infected animals that received either 0.5×10^5 FL-DC or GM-DC prior to infection was assessed by performing CFU counts on lung homogenates on day 21 post infection. * $p<0.05$ versus control *B. pertussis* infected mice. *D*, The number of $CD3^+CD4^+$, $CD3^+CD8^+$, $B220^+$ and $GR1^+$ cells in lung homogenates from *B. pertussis*-infected mice and infected animals that received FL-DC were determined 7, 14 and 21 days after infection. *E*, The number of $CD11c^+$ and $CD11c^+CD8\alpha^+$ DC infiltrating the lungs 7 days post challenge in control infected mice and mice that received sorted FL-DC. Results are representative of 3 separate experiments. * $p<0.05$ versus control *B. pertussis* infected mice.

FIGURE 9. Blocking CD103 function *in vivo* impairs bacterial clearance. Mice were infected with 2×10^{10} live, virulent *B. pertussis*. Infected lungs and CLN were removed and

digested at various time points post infection. The percentage (*A*), based on the viable lymphocyte gate and total (*B*) number of $CD11c^{+}CD8\alpha^{+}CD103^{+}$ DC in CLN from non-infected control mice and in CLN and lungs from infected animals, determined by flow cytometric analysis daily, up to day 7 after challenge with *B. pertussis*. * $p<0.05$, ** $p<0.01$, versus un-infected control mice. *C-D*, Mice were injected with 50 μg of either anti-CD103 or rat anti-HRP IgG_1 antibody (Control) i.p. 24 h prior to infection in order to block CD103 function in vivo. Bacterial clearance (*C*) and cellular infiltration into the lung (*D*) was examined on day 21 after *B. pertussis* infection. Results are representative of 2 separate experiments. ** $p<0.01$, versus non-depleted *B. pertussis* infected control mice.

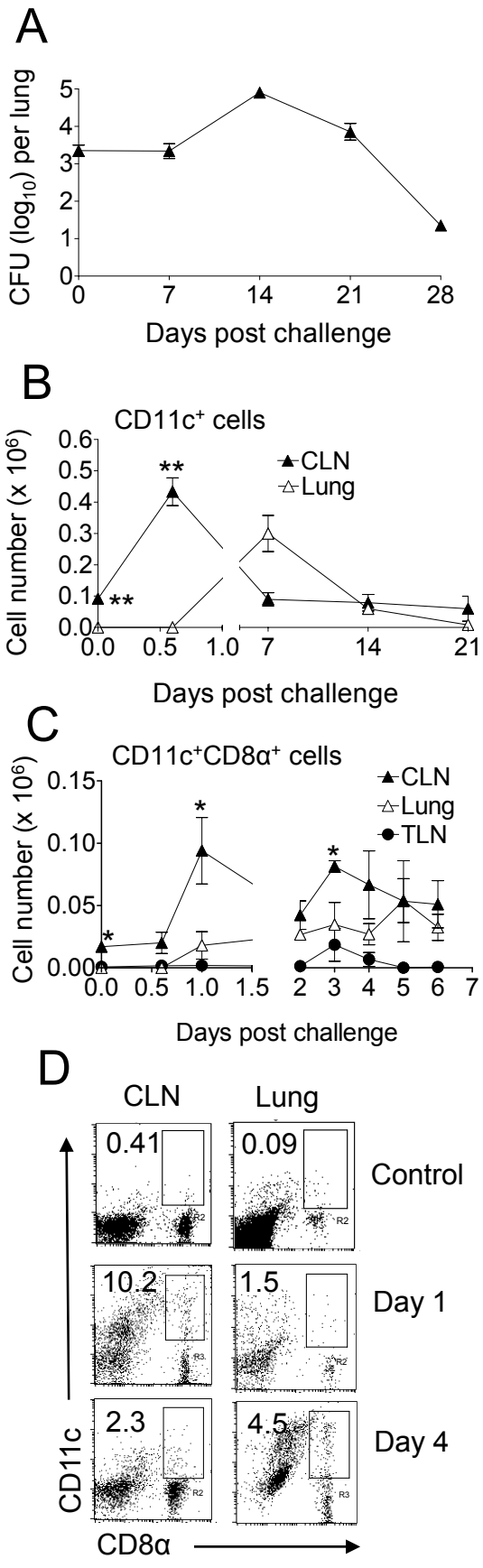
A
CFU (log10) per lung
0
1
2
3
4
5
0
7
14
21
28
Days post challenge
B
CD11c+ cells
Cell number (x 10^6)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
**
**
CLN
Lung
0.0
0.5
1.0
7
14
21
Days post challenge
C
CD11c+CD8α+ cells
Cell number (x 10^6)
0.00
0.05
0.10
0.15
*
*
*
CLN
Lung
TLN
0.0
0.5
1.0
1.5
2
3
4
5
6
7
Days post challenge
D
CLN
Lung
0.41
0.09
Control
10.2
1.5
Day 1
2.3
4.5
Day 4
CD11c
CD8α

Figure 1

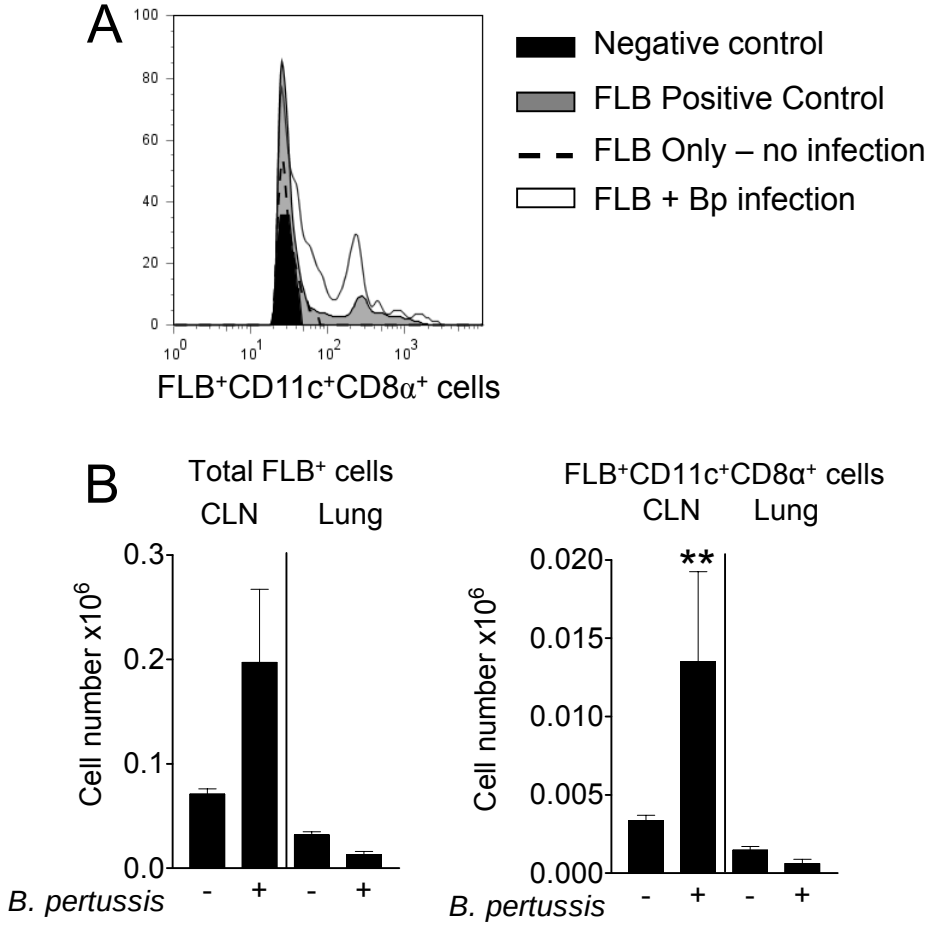
A
Negative control
FLB Positive Control
FLB Only – no infection
FLB + Bp infection
FLB+CD11c+CD8α+ cells
B
Total FLB+ cells
CLN
Lung
Cell number x10^6
B. pertussis
FLB+CD11c+CD8α+ cells
CLN
Lung
**
Cell number x10^6
B. pertussis

Figure 2

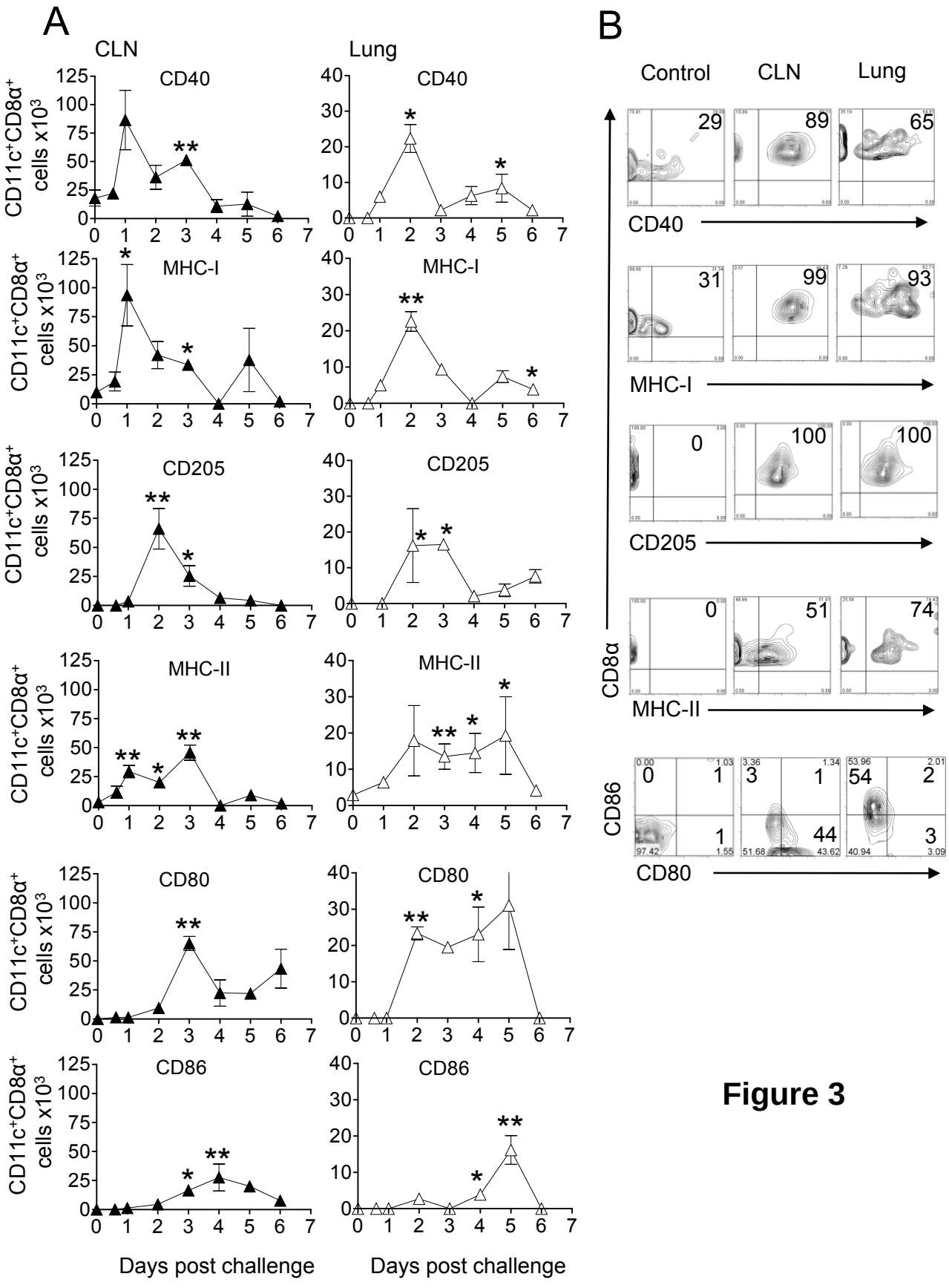
A
CLN
Lung
CD40
MHC-I
CD205
MHC-II
CD80
CD86
CD11c+CD8α+ cells x10³
Days post challenge
B
Control
CLN
Lung
CD40
MHC-I
CD205
MHC-II
CD86
CD8α
CD80

Figure 3

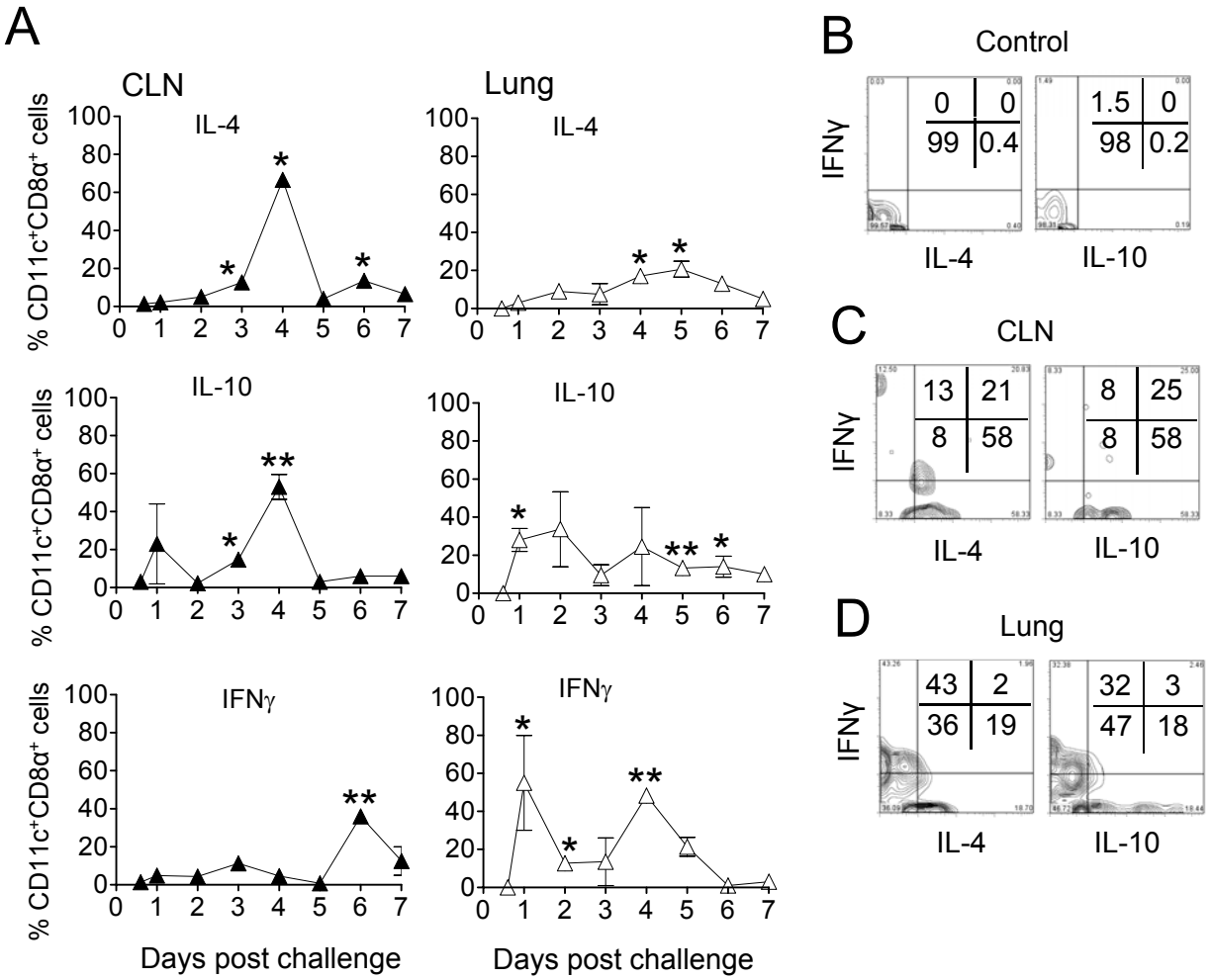
A
CLN
Lung
IL-4
IL-10
IFNγ
% CD11c+CD8α+ cells
Days post challenge
B
Control
IFNγ
IL-4
IL-10
0
0
99
0.4
1.5
0
98
0.2
C
CLN
13
21
8
58
8
25
8
58
D
Lung
43
2
36
19
32
3
47
18

Figure 4

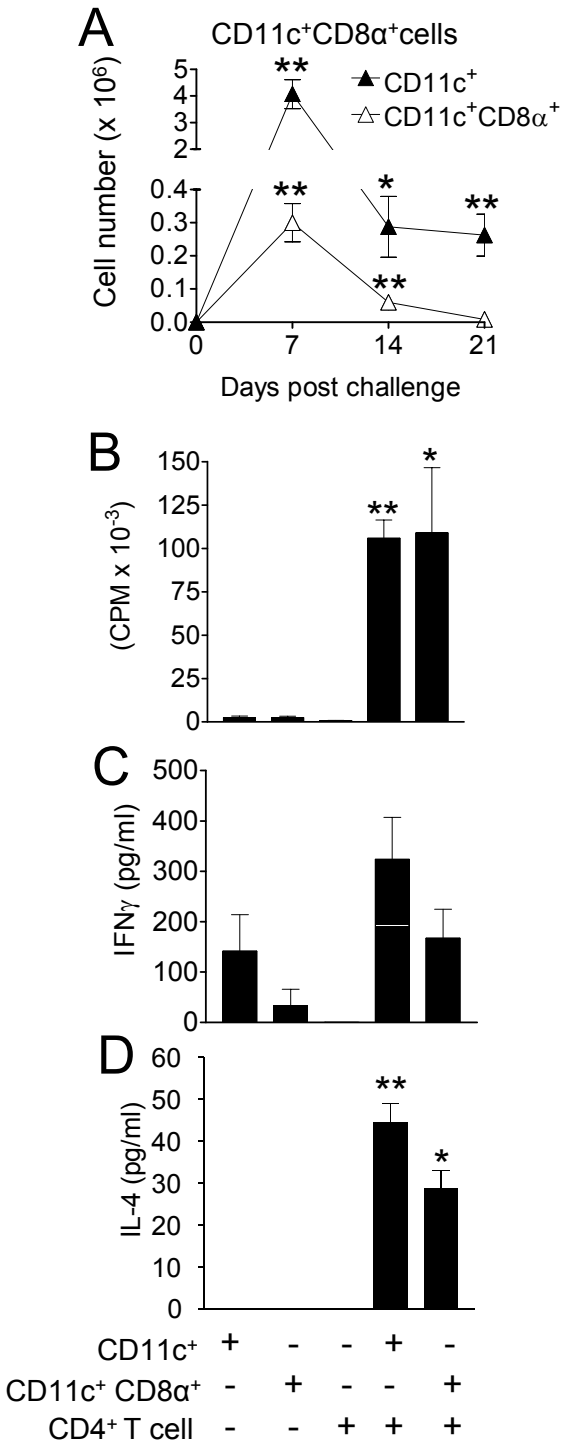
A
CD11c+CD8α+cells
Cell number (x 10^6)
CD11c+
CD11c+CD8α+
Days post challenge
B
(CPM x 10^-3)
C
IFNγ (pg/ml)
D
IL-4 (pg/ml)
CD11c+
CD11c+ CD8α+
CD4+ T cell

Figure 5

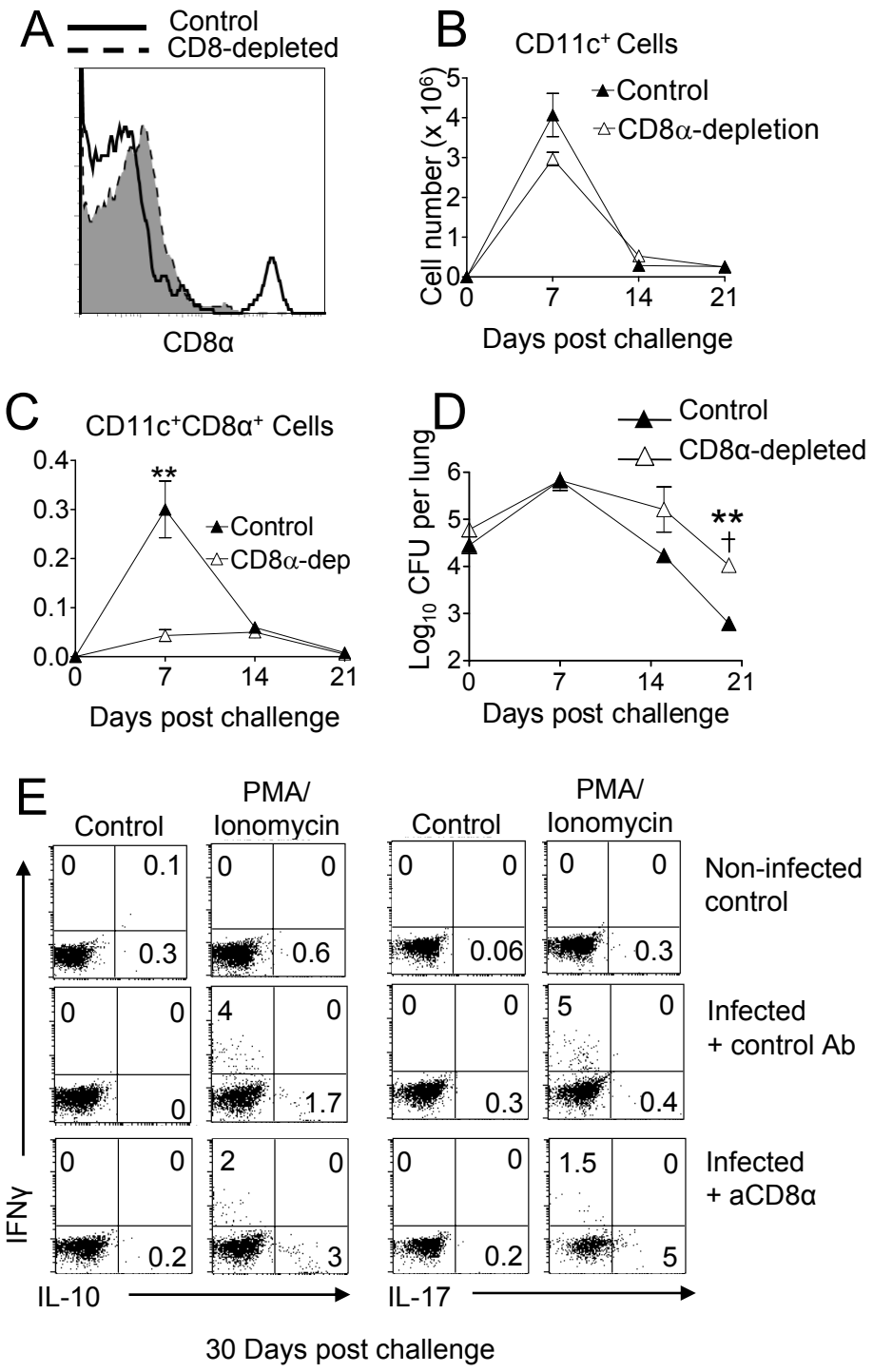
A
Control
CD8-depleted
CD8α
B
CD11c+ Cells
Control
CD8α-depletion
Cell number (x 10^6)
Days post challenge
C
CD11c+CD8α+ Cells
Control
CD8α-dep
Days post challenge
D
Control
CD8α-depleted
Log10 CFU per lung
Days post challenge
E
Control
PMA/ Ionomycin
Control
PMA/ Ionomycin
Non-infected control
Infected + control Ab
Infected + aCD8α
IFNγ
IL-10
IL-17
30 Days post challenge

Figure 6

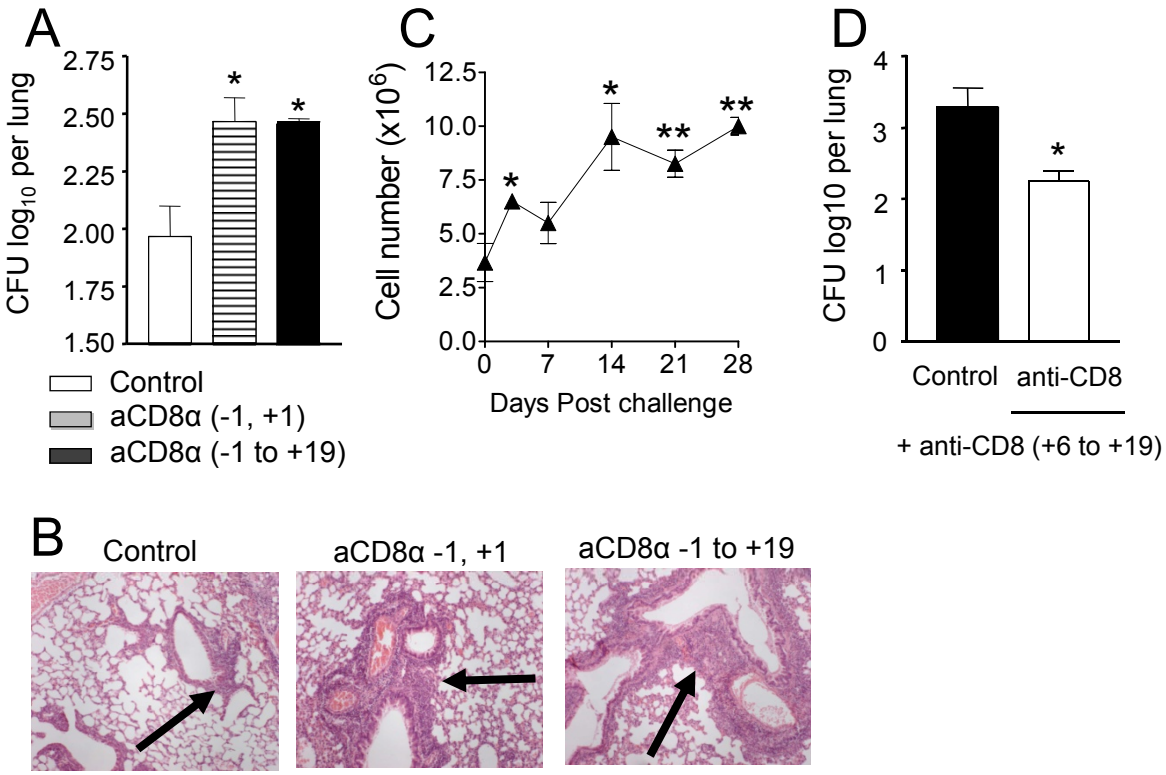
A
CFU log10 per lung
2.75
2.50
2.25
2.00
1.75
1.50
*
*
Control
aCD8α (-1, +1)
aCD8α (-1 to +19)
C
Cell number (x10^6)
12.5
10.0
7.5
5.0
2.5
0.0
0
7
14
21
28
Days Post challenge
D
CFU log10 per lung
4
3
2
1
0
Control
anti-CD8
+ anti-CD8 (+6 to +19)
B
Control
aCD8α -1, +1
aCD8α -1 to +19

Figure 7

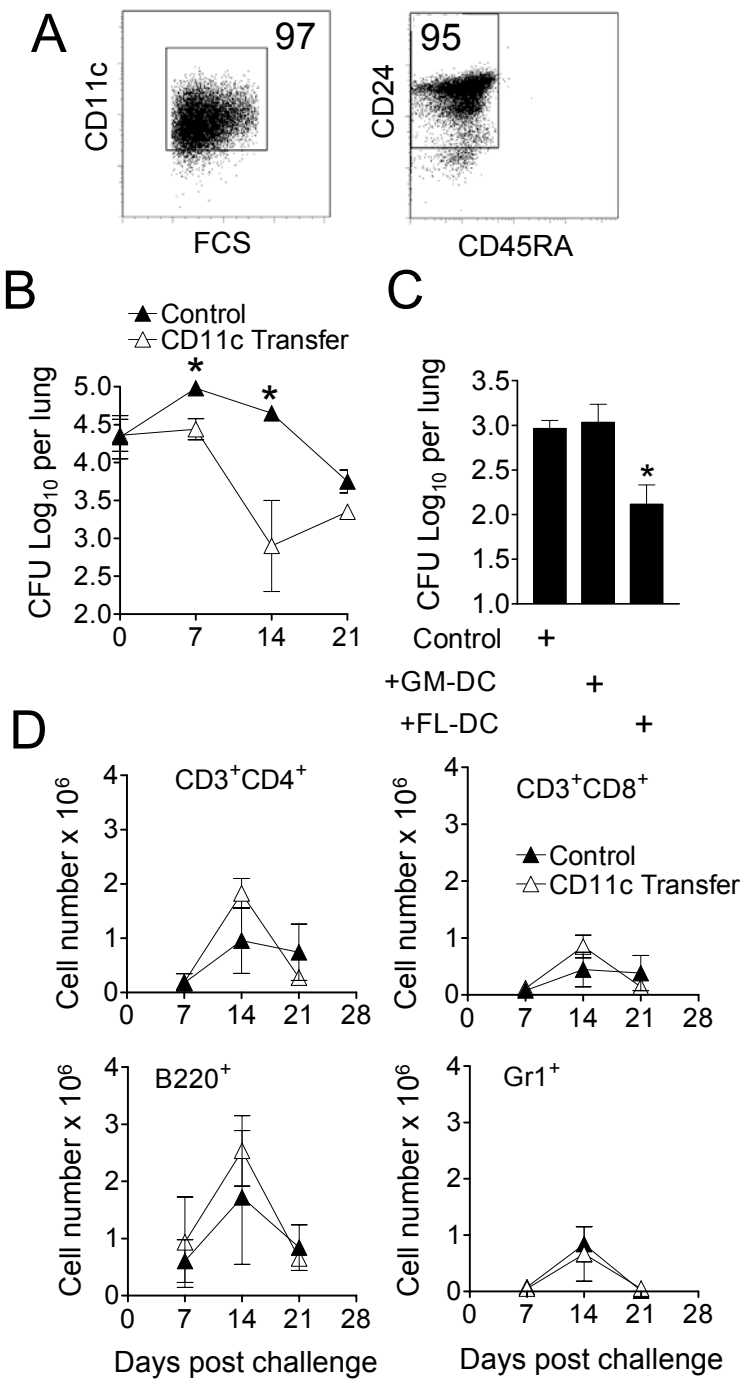
A
97
CD11c
FCS
95
CD24
CD45RA
B
Control
CD11c Transfer
CFU Log10 per lung
C
CFU Log10 per lung
Control +
+GM-DC +
+FL-DC +
D
CD3+CD4+
CD3+CD8+
Control
CD11c Transfer
B220+
Gr1+
Cell number x 10^6
Days post challenge
E
CD11C+
CD11C+CD8α+
Cell number x 10^6
Control
FL-DC transfer

Figure 8

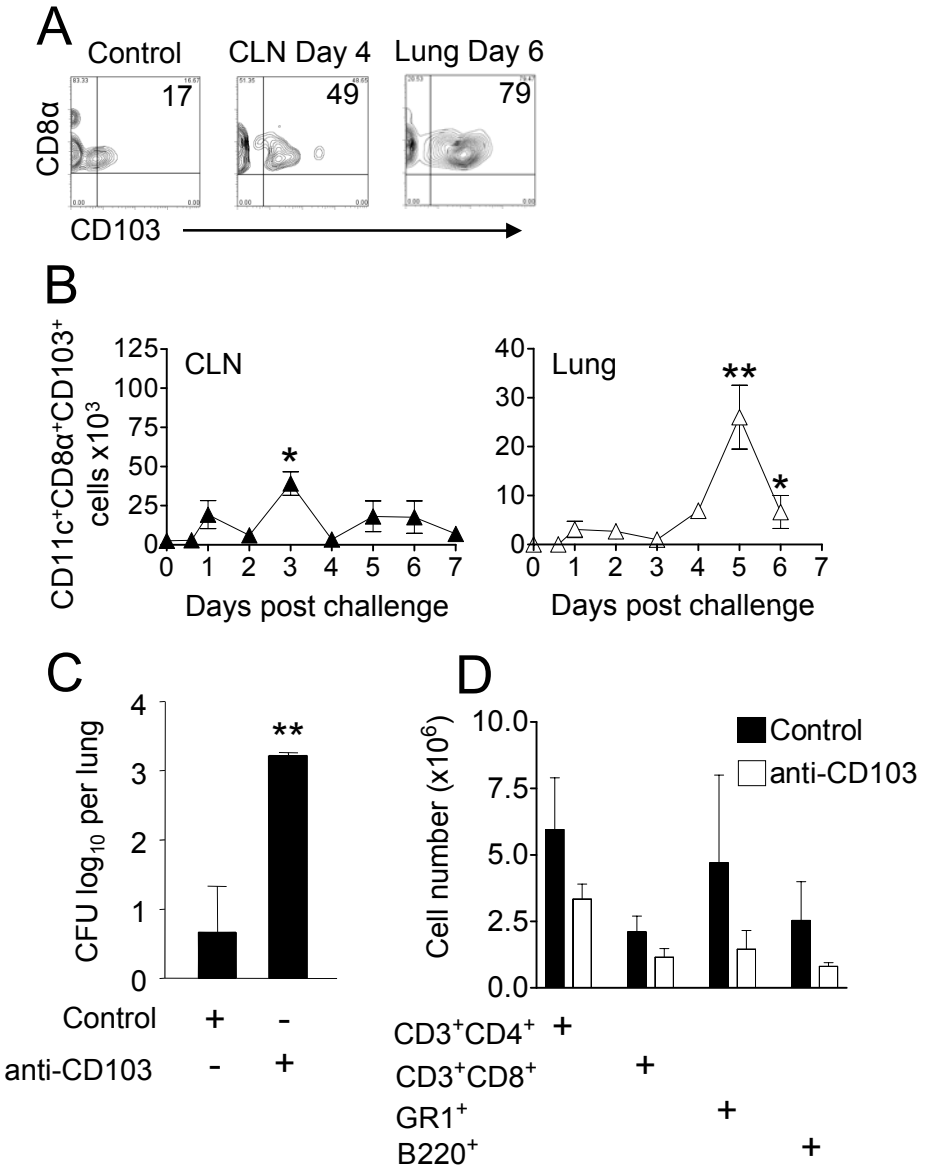
A
Control
CLN Day 4
Lung Day 6
17
49
79
CD8α
CD103
B
CD11c+CD8α+CD103+ cells x10³
CLN
Lung
Days post challenge
Days post challenge
C
CFU log10 per lung
Control
anti-CD103
D
Cell number (x10⁶)
Control
anti-CD103
CD3+CD4+
CD3+CD8+
GR1+
B220+

Figure 9