nature immunology

IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge

Shabaana A Khader[1], Guy K Bell[1], John E Pearl[1], Jeffrey J Fountain[1], Javier Rangel-Moreno[1], Garth E Cilley[1], Fang Shen[2], Sheri M Eaton[1], Sarah L Gaffen[2], Susan L Swain[1], Richard M Locksley[3], Laura Haynes[1], Troy D Randall[1] & Andrea M Cooper[1]

Interferon-γ is key in limiting *Mycobacterium tuberculosis* infection. Here we show that vaccination triggered an accelerated interferon-γ response by CD4+ T cells in the lung during subsequent *M. tuberculosis* infection. Interleukin 23 (IL-23) was essential for the accelerated response, for early cessation of bacterial growth and for establishment of an IL-17-producing CD4+ T cell population in the lung. The recall response of the IL-17-producing CD4+ T cell population occurred concurrently with expression of the chemokines CXCL9, CXCL10 and CXCL11. Depletion of IL-17 during challenge reduced the chemokine expression and accumulation of CD4+ T cells producing interferon-γ in the lung. We propose that vaccination induces IL-17-producing CD4+ T cells that populate the lung and, after challenge, trigger the production of chemokines that recruit CD4+ T cells producing interferon-γ, which ultimately restrict bacterial growth.

Tuberculosis is a leading cause of morbidity and mortality in the world, with 8 million new cases and 2 million deaths annually[1]. Improved control of tuberculosis requires improvement of the vaccine now in use, bacille Calmette-Guerin, which is of limited value. People vaccinated with bacille Calmette-Guerin show strong cellular responses to *Mycobacterium tuberculosis*[2], but protection from natural, aerosol infection is highly variable[3]. In animal models of vaccination, the recall response is not detected in the lung until 15 d after aerosol challenge; this delay allows bacterial growth to occur[4,5]. Development of a vaccine that can overcome this delay requires elucidation of the molecular and cellular events underlying the recall response to aerosol challenge with *M. tuberculosis*.

The importance of interferon-γ (IFN-γ)–producing CD4+ T cells in primary resistance to *M. tuberculosis* has been established[6]. Furthermore, cytokines linked to the development of T helper type 1 (T_H1) cells, such as interleukin 12 (IL-12), are involved in primary resistance in humans[7,8] and in animal models[9–11]. IFN-γ and IL-12 have also been linked to recall responses in vaccinated mice[4,5,12,13]. In contrast, the related cytokine, IL-23, is not involved in primary resistance to *M. tuberculosis* even though IL-23 is required for the generation of an IL-17-producing, mycobacteria-specific CD4+ T cell response[14,15]. IL-17-producing cells (T_H-17 cells) represent a T helper lineage that requires transforming growth factor-β (TGF-β)[16–18] and IL-6 (ref. 18) for initiation and IL-23 (refs. 17,19,20) to become an established population. T_H-17 cells are associated with autoimmune disease[20–22], mediate neutrophil recruitment, participate in inflammatory responses[19,23–27] and are negatively regulated by IFN-γ[15,28,29]. Despite the lack of involvement of IL-23 in primary resistance to *M. tuberculosis*, the fact that IL-23 acts on T cells with an activated or memory phenotype[20,30–32] suggests that this cytokine may be involved in recall responses.

To investigate the involvement of IL-23 in CD4+ T cell recall responses, we used a defined molecular vaccine to induce epitope-specific, long-lived CD4+ T cells without inducing responses to other mycobacterial proteins. Vaccination elicited antigen-specific T cells producing IFN-γ and T cells producing that persisted in the central lymphoid organs and antigen-specific T cells producing IL-17 that persisted in the lung. Establishment of persistent IL-17-producing T cells required IL-23. After challenge, the IL-17 recall response preceded the IFN-γ recall response, and the accelerated IFN-γ response and lower bacterial burden in vaccinated wild-type mice was not present in IL-23-deficient mice. Treatment with exogenous IL-17 restored the IFN-γ recall response in IL-23-deficient mice, whereas depletion of IL-17 in vaccinated wild-type mice reduced the response. We propose that vaccination results in the generation of T cells producing IFN-γ or IL-17, and that T cells producing IL-17 populate the lung and respond rapidly to infection. This release of IL-17 promotes chemokine expression in the lung

[1]Trudeau Institute, Saranac Lake, New York 12983, USA. [2]Department of Oral Biology, School of Dental Medicine, University at Buffalo, The State Univeristy of New York, Buffalo, New York 14214, USA. [3]Howard Hughes Medical Institute, Department of Medicine and Microbiology & Immunology, University of California San Francisco, San Francisco, California 94143, USA. Correspondence should be addressed to A.M.C. (acooper@trudeauinstitute.org).

Received 12 December 2006; accepted 7 February 2007; published online 11 March 2007; doi:10.1038/ni1449

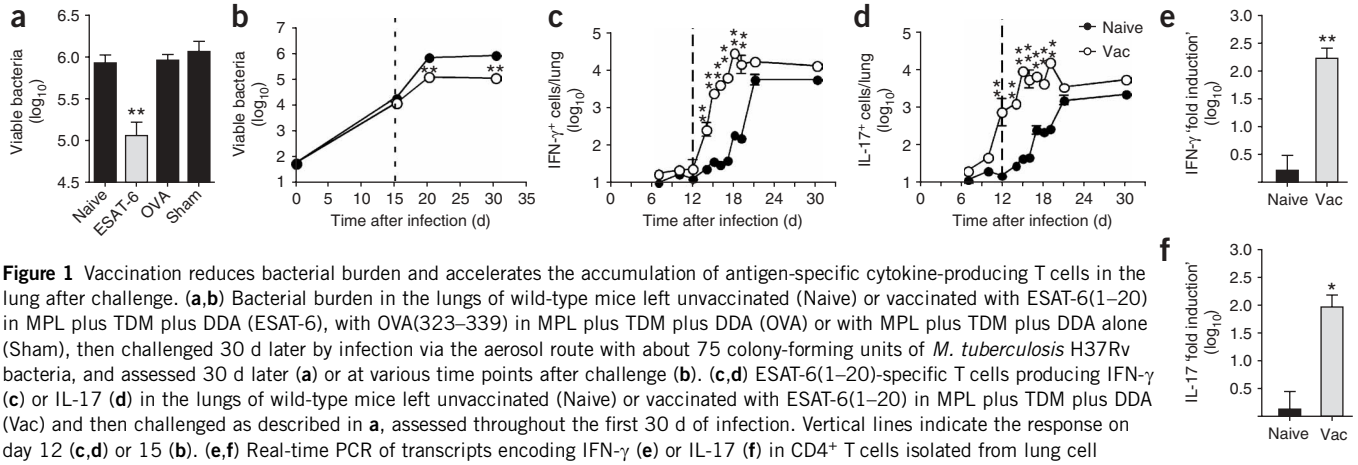

Figure 1 Vaccination reduces bacterial burden and accelerates the accumulation of antigen-specific cytokine-producing T cells in the lung after challenge. (**a**,**b**) Bacterial burden in the lungs of wild-type mice left unvaccinated (Naive) or vaccinated with ESAT-6(1–20) in MPL plus TDM plus DDA (ESAT-6), with OVA(323–339) in MPL plus TDM plus DDA (OVA) or with MPL plus TDM plus DDA alone (Sham), then challenged 30 d later by infection via the aerosol route with about 75 colony-forming units of *M. tuberculosis* H37Rv bacteria, and assessed 30 d later (**a**) or at various time points after challenge (**b**). (**c**,**d**) ESAT-6(1–20)-specific T cells producing IFN-γ (**c**) or IL-17 (**d**) in the lungs of wild-type mice left unvaccinated (Naive) or vaccinated with ESAT-6(1–20) in MPL plus TDM plus DDA (Vac) and then challenged as described in **a**, assessed throughout the first 30 d of infection. Vertical lines indicate the response on day 12 (**c**,**d**) or 15 (**b**). (**e**,**f**) Real-time PCR of transcripts encoding IFN-γ (**e**) or IL-17 (**f**) in CD4$^+$ T cells isolated from lung cell suspensions from mice vaccinated and infected as described in **c**,**d** collected 15 d after infection. *, $P \leq 0.05$, and **, $P \leq 0.01$, versus naive (Student's *t*-test). Data are one representative of a total of four experiments (**a**,**b**; mean + s.d. of four mice per group; some error bars are covered by symbols in **b**), or one experiment for total kinetics (values at day 12 and day 15 are representative of results from three other independent experiments), with four mice (**c**,**d**), or one representative of a total of two experiments (**e**,**f**; mean + s.d. relative to uninfected naive mice; $n = 3$ samples each containing pooled cells from four mice per group).

and the recruitment of T cells producing IFN-γ, which stop bacterial growth.

RESULTS

Vaccine-induced T cell responses after challenge

To investigate the ability of vaccination to elicit a protective immune response to *M. tuberculosis*, we vaccinated mice with an I-A^b-restricted peptide comprising amino acids 1–20 of the *M. tuberculosis* 6-kilodalton early secreted antigenic target protein (ESAT-6(1–20)), which is recognized early during *M. tuberculosis* infection[33], in an adjuvant composed of MPL (monophosphoryl lipid A), a Toll-like receptor 4 stimulant[34], TDM (trehalose dicorynomycolate), which acts through pathway dependent on the MyD88 adaptor protein[35], and the small cationic molecule DDA (dimethyl dioctadecylammonium bromide), which acts as an antigen 'depot'. Vaccination resulted in protection against aerosol challenge with *M. tuberculosis*; this protective effect depended on the presence of the *M. tuberculosis* peptide ESAT-6(1–20) (**Fig. 1a**). Vaccination suppressed the growth of the bacterial population between days 15 and 20 (**Fig. 1b**), similar to the protection reported before[4,5]. These data demonstrated that vaccination with a single mycobacterial peptide in the appropriate adjuvant was sufficient to mediate protection against aerosol *M. tuberculosis* challenge.

We next compared the T cell responses in the lungs of vaccinated and unvaccinated mice after aerosol challenge with *M. tuberculosis*. We noted accelerated accumulation of ESAT-6(1–20)-specific IFN-γ-producing T cells in vaccinated mice beginning on day 15 after infection (**Fig. 1c**). Notably, IL-17-producing ESAT-6(1–20)-specific T cells accumulated in the lung beginning at day 12 (**Fig. 1d**), 3 d before the accumulation of IFN-γ-producing cells. By 15 d after infection, CD4$^+$ T cells purified from the lungs of vaccinated mice expressed more *Ifng* transcripts (**Fig. 1e**) and *Il17a* transcripts (**Fig. 1f**) than did CD4$^+$ T cells isolated from the lungs of unvaccinated mice. Thus, during the recall response to aerosol *M. tuberculosis* challenge, IL-17-producing cells accumulated more rapidly in the lung than did IFN-γ-producing T cells.

Cytokines in vaccine-induced protection

As IL-12 (composed of IL-12p40 and IL-12p35 subunits) is required for the generation of IFN-γ-producing CD4$^+$ T cells, and IL-23 (composed of IL-12p40 and IL-23p19 subunits) is required for generation of IL-17-producing CD4$^+$ T cells[14], we sought to test the function of each cytokine in recall responses. Therefore, we vaccinated wild-type mice, IL-12p35-deficient mice and IL-12p40-deficient mice with ESAT-6(1–20) in adjuvant, waited 30 d and challenged the mice with *M. tuberculosis* by the aerosol route. Vaccination elicited a protective response in wild-type and IL-12p35-deficient mice but not in IL-12p40-deficient mice (**Fig. 2a**). Furthermore, the accumulation of IFN-γ-producing and IL-17-producing T cells was accelerated

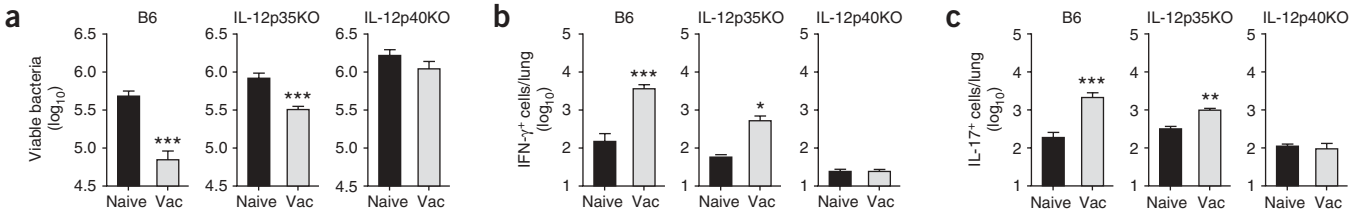

Figure 2 IL-12p40 but not IL-12p35 is essential for vaccine-induced reduced bacterial burden and the accelerated accumulation of antigen-specific cytokine-producing T cells in the lungs. Wild-type (B6), IL-12p35-deficient (IL-12p35KO) or IL-12p40-deficient (IL-12p40KO) mice were left naive or were vaccinated and then challenged as described in **Figure 1a**,**b**. (**a**) Bacterial burden in the lungs at 30 d after challenge. (**b**,**c**) ELISPOT analysis of the number of ESAT-6(1–20)-specific IFN-γ-producing cells (**b**) and IL-17-producing cells (**c**) in the lungs at 15 d after challenge. *, $P \leq 0.01$, **, $P \leq 0.001$, and ***, $P \leq 0.0001$, versus naive (Student's *t*-test). Data (mean + s.d. of four mice per group) are one representative of three (wild-type) or Two (IL-12p35-deficient and IL-12p40-deficient) independent experiments.

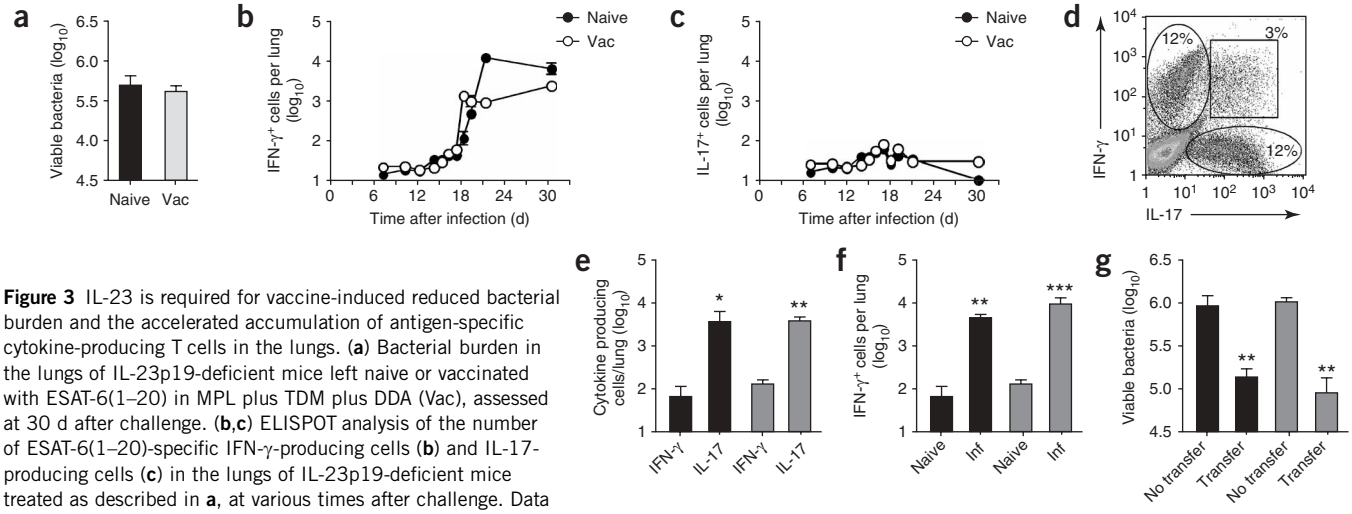

Figure 3 IL-23 is required for vaccine-induced reduced bacterial burden and the accelerated accumulation of antigen-specific cytokine-producing T cells in the lungs. (**a**) Bacterial burden in the lungs of IL-23p19-deficient mice left naive or vaccinated with ESAT-6(1–20) in MPL plus TDM plus DDA (Vac), assessed at 30 d after challenge. (**b**,**c**) ELISPOT analysis of the number of ESAT-6(1–20)-specific IFN-γ-producing cells (**b**) and IL-17-producing cells (**c**) in the lungs of IL-23p19-deficient mice treated as described in **a**, at various times after challenge. Data (mean + s.d. for four mice per group (**a**–**c**); some error bars are covered by symbols in **b**,**c**) are one representative experiment of three independent experiments (**a**) or represent one experiment for total kinetics or results from six experiments for values at day 15 (**b**,**c**). (**d**) Intracellular staining and flow cytometry of the frequency of cytokine-producing $CD4^+$ T cells from wild-type mice vaccinated as described in **a**; cells from draining lymph nodes were grown *in vitro* with peptide and IL-23 before analysis. (**e**) ELISPOT assay of peptide-specific T cells producing IFN-γ or IL-17 in the lung at day 15 after transfer of $CD4^+$ T cells (purified from cultures generated as described in **d**) into wild-type host mice (black bars) and IL-23p19-deficient host mice (gray bars). (**f**) ELISPOT analysis of ESAT-6(1–20)-specific IFN-γ-producing T cells in the lungs of wild-type (black bars) and IL-23p19-deficient (gray bars) host mice generated as described in **e**, allowed to 'rest' for 15 d and then left naive or infected (Inf) as described in **Figure 1a**,**b**. (**g**) Bacterial burden in recipient mice (Transfer) generated as described in **e**, and unmanipulated mice (No transfer), infected as described in **Figure 1a**,**b** and assessed at day 30 after challenge in wild-type host mice (black bars) and IL-23p19-deficient host mice (gray bars). *, $P \leq 0.05$, **, $P \leq 0.001$, and ***, $P \leq 0.0001$, relative to IFN-γ^+ (**e**), naive (**f**) or No transfer (**g**; Student's *t*-test). Data are one representative of three independent cultures done twice (**d**) or one representative of two independent experiments (**e**–**g**; mean + s.d. of four mice per group).

in the lungs of vaccinated wild-type and IL-12p35-deficient mice but not in those of IL-12p40-deficient mice by day 15 (**Fig. 2b**,**c**). These data demonstrated that IL-12p40 but not IL-12p35 was essential for the vaccine-induced protective recall response.

We next investigated the function of IL-23 in the recall response and found that in contrast to the situation in wild-type mice (**Fig. 1**), vaccination of IL-23-deficient mice did not lower the bacterial burden in the lung 30 d after challenge and failed to elicit an accelerated IFN-γ or IL-17 T cell recall response (**Fig. 3a**–**c**). These data showed that IL-23 but not IL-12 was essential for the induction of recall responses and protection. To determine whether IL-23 was required during challenge, we removed draining lymph nodes from wild-type mice 10 d after vaccination, grew $CD4^+$ T cells *in vitro* with ESAT-6(1–20) and IL-23, and transferred those $CD4^+$ T cells into naive wild-type or IL-23-deficient host mice. The transferred $CD4^+$ T cell population had equivalent frequencies of cells producing IFN-γ- and IL-17, but by day 15 after transfer, recipient mice had 16-fold more antigen-specific IL-17-producing $CD4^+$ T cells than antigen-specific IFN-γ-producing $CD4^+$ T cells in the lung (**Fig. 3d**,**e**). Recipient mice challenged with *M. tuberculosis* by the aerosol route on day 15 after transfer showed rapid accumulation of ESAT-6(1–20)-specific IFN-γ-producing $CD4^+$ T cells (**Fig. 3f**). Recipient mice were also protected and had a lower bacterial burden even in the absence of IL-23 (**Fig. 3g**). These data suggested that IL-23 was not directly required for the recall response.

Vaccination-induced antimycobacterial function

To determine how the absence of IL-23 affects the ability of vaccination to protect against challenge infection, we compared the accumulation of activated effector cells and antimycobacterial agents in granulomatous structures in the lungs of vaccinated wild-type and cytokine-deficient mice. At 15 d after aerosol challenge with *M. tuberculosis*, more $CD4^+$ T cells with an activated $CD44^{hi}CXCR3^+$ phenotype[36] were present in the lungs of vaccinated wild-type than in those of vaccinated IL-23p19-deficient mice (**Fig. 4a**–**c**). Large mononuclear granulomatous structures were present in vaccinated wild-type but not IL-23p19-deficent mice (**Fig. 4d**). The localization of activated $CD4^+$ T cells together with myeloid cells in granulomas coincided with the vaccine-induced accelerated upregulation of major histocompatibility complex (MHC) class II on $CD11c^+$ dendritic cells (DCs) in the lungs of vaccinated wild-type and IL-12p35-deficient mice but not on DCs from the lungs of vaccinated mice deficient in either IFN-γ or IL-12p40 (**Fig. 4e**). Upregulation of MHC class II was detectable 15 d after challenge in vaccinated mice and 21 d after challenge in unvaccinated mice. Finally, vaccination-induced antimycobacterial responses occurred rapidly in the lungs of wild-type mice, as demonstrated by the higher expression of inducible nitric oxide synthase on lung macrophages (**Fig. 4f**) and by the significant increase in mRNA encoding the LRG-47 antimycobacterial molecule[37] by day 15 (**Supplementary Fig. 1** online). These data demonstrated that the vaccine-induced antimycobacterial function in the lung was temporally associated with the accumulation of activated IFN-γ-producing $CD4^+$ T cells.

Vaccine-induced chemokine expression

The failure of vaccinated IL-23p19-deficient mice to generate granulomas and to accumulate activated $CXCR3^+CD4^+$ T cells in the lung (**Fig. 4**) indicated a recruitment deficiency in the recall response to the lung. As chemokines mediate cell recruitment, we compared the induction of chemokine transcripts in the lungs of vaccinated wild-type and IL-23p19-deficient mice after challenge. In wild-type but not IL-23p19-deficient mice, vaccination triggered expression of the genes encoding the CXCR3 ligands CXCL9, CXCL10 and CXCL11 at day 12 after infection (**Fig. 5a**). The expression of these chemokines

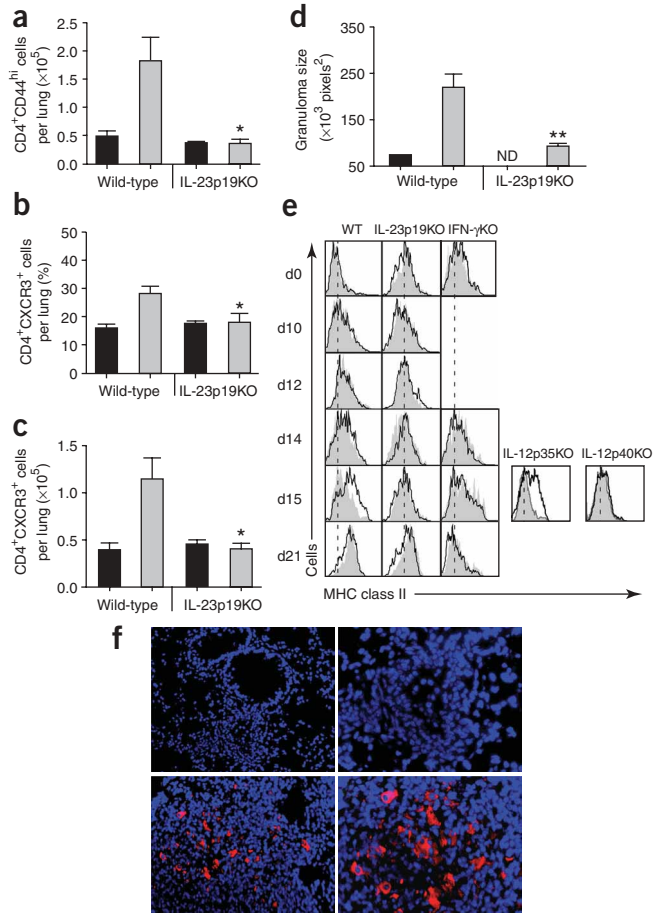

Figure 4 Cellular responses in the lung are accelerated in vaccinated mice. Wild-type mice and IL-23p19-deficient mice (IL-23p19KO) were vaccinated and challenged as described in **Figure 1a**,**b**. (**a**–**c**) Flow cytometry of the number of $CD4^+CD44^{hi}$ cells (**a**) and the frequency (**b**) and number (**c**) of $CD4^+CXCR3^+$ T cells in the lungs of unvaccinated mice (black bars) and vaccinated mice (gray bars) 15 d after infection. (**d**) Morphometric analysis of average granuloma size in the lungs unvaccinated mice (black bars) or vaccinated mice (gray bars). ND, not detected. *, $P \leq 0.05$, and **, $P \leq 0.01$, relative to wild-type vaccinated (Student's *t*-test). Data (**a**–**d**; mean + s.d. of four mice per group) are one representative of two independent experiments. (**e**) MHC class II on $CD11c^+$ cells with low autofluorescence from the lungs of unvaccinated mice (filled histograms) or vaccinated mice (black lines) at various times after infection (days 0–21 (d0–d21)). Dashed vertical lines, expression at day 0. Data are representative of one of four mice analyzed in two independent experiments. (**f**) Lung sections from unvaccinated (top row) and vaccinated (bottom row) wild-type mice at 15 d after infection, stained for expression of inducible nitric oxide synthase. Original magnification, ×20 (left) or ×40 (right). Representative of four lungs per group analyzed in two independent experiments.

corresponded with the accumulation of IL-17-producing antigen-specific T cells (**Fig. 1**) and the expression of *Il17a* but not *Ifng* or *Tnf* mRNA (**Fig. 5a**) in the lungs of vaccinated wild-type mice. To determine whether IL-17 induced the chemokines, we used computational analysis of transcription factor–binding sites to show that the promoters of *Cxcl9*, *Cxcl10* and *Cxcl11* contain transcription factor–binding patterns that resemble patterns associated with IL-17-responsive genes[38] (data not shown). Furthermore, treatment with IL-17-neutralizing antibody on day 12 after challenge significantly inhibited induction of the genes encoding all three chemokines in vaccinated wild-type mice (**Fig. 5b**). Administration of antibody to IL-17 (anti-IL-17) on d 12 and 14 after challenge also significantly reduced the frequency of antigen-specific IFN-γ-producing T cells in the lungs of vaccinated wild-type mice (**Fig. 5c**).

Finally, to determine whether IL-17 was able to restore the IFN-γ recall response in the lungs of vaccinated IL-23p19-deficient mice, we delivered exogenous IL-17 to these mice on day 12 after challenge, the time when vaccinated wild-type mice showed an IL-17 recall response. Exogenous IL-17 restored the accelerated accumulation of IFN-γ-producing $CD4^+$ T cells in the lungs of vaccinated but not unvaccinated IL-23p19-deficient mice (**Fig. 5d**). These data showed that the accelerated chemokine response after challenge in the lungs of

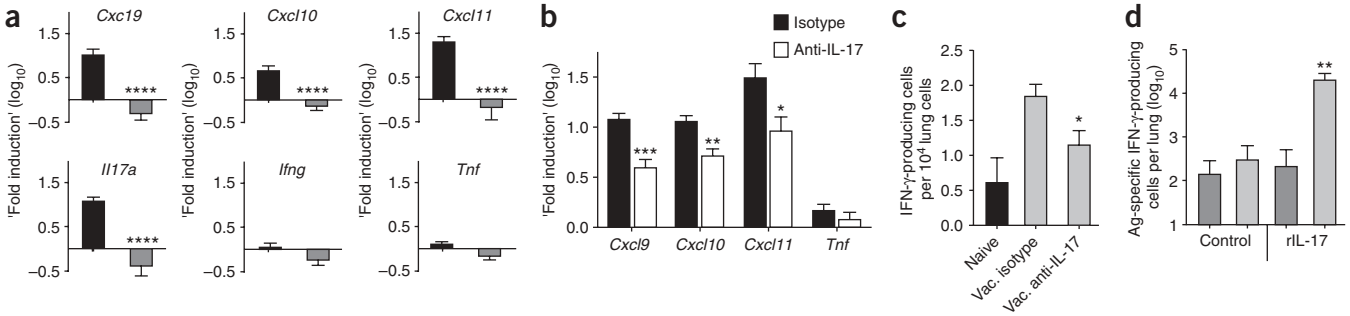

Figure 5 Vaccinated mice show early IL-17- and IL-23-dependent chemokine production. (**a**) RT-PCR of gene expression in lung tissues from wild-type mice (black bars) and IL-23p19-deficient mice (gray bars), vaccinated and infected as described in **Figure 1a**,**b**; tissues were collected on day 12 after challenge and assessed relative to uninfected vaccinated wild-type or IL-23p19-deficient tissue. (**b**) RT-PCR of the induction of chemokine transcripts in wild-type mice vaccinated and infected as described in **Figure 1a**,**b** and then treated with isotype control antibody or anti-IL-17 on day 12 after infection; lung tissue was isolated on day 13 for analysis of induction relative to that in uninfected vaccinated mice. (**c**) ELISPOT analysis of the frequency of ESAT-6(1–20)-specific IFN-γ-producing cells in wild-type mice vaccinated and challenged as described in **Figure 1a**,**b** and treated on days 12 and 14 with isotype control antibody or anti-IL-17, assessed at day 16 after challenge. (**d**) ELISPOT analysis of the frequency of ESAT-6(1–20)-specific (Ag-specific) IFN-γ-producing cells in IL-23p19-deficient mice left naive (dark gray bars) or vaccinated (light gray bars) and challenged as described in **Figure 1a**,**b** and then injected intratracheally with saline (Control) or recombinant IL-17 (rIL-17) on day 12 after infection, assessed at day 15 after infection. *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$, and ****, $P \leq 0.0001$, versus wild-type vaccinated (**a**), isotype (**b**), vaccinated, isotype-treated (**c**) or vaccinated, saline-treated (**d**; Student's *t*-test). Data (mean + s.d.) are one representative of two independent experiments with four (**a**) or five (**b**–**d**) mice.

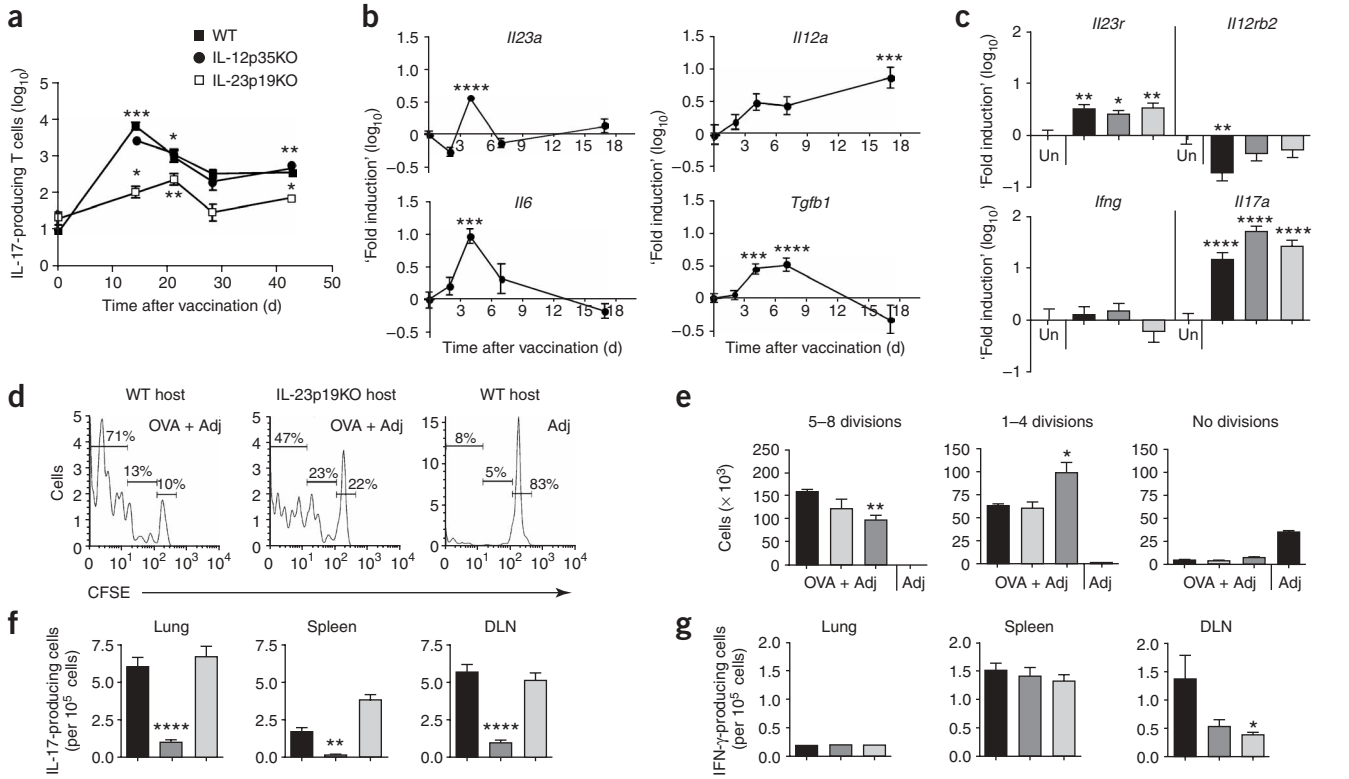

Figure 6 Vaccination-induced IL-23-dependent lung IL-17-producing CD4$^+$ T cell population. (**a**) ELISPOT analysis of the number of peptide-specific IL-17-producing T cells in the draining lymph nodes of mice vaccinated with ESAT-6(1–20) in MPL plus TDM plus DDA. *, $P \leq 0.05$, **, $P \leq 0.01$, and ***, $P \leq 0.001$, relative to day 0 for wild-type (WT) or IL-23p19-deficient (Student's *t*-test). The response of vaccinated IL-23p19-deficient mice was also significantly less than that of wild-type mice on days 14 ($P \leq 0.001$), 21 ($P \leq 0.05$) and 42 ($P \leq 0.01$). Values were not obtained for IL-12p35-deficient mice at day 0; however, historical values ($n = 8$ mice in two other experiments) were equivalent to values for wild-type and IL-23p19-deficient mice at day 0. (**b**) RT-PCR of gene expression by CD11c$^+$YFP$^+$ cells from draining lymph nodes of B6.yet40 mice vaccinated as described in **a**. (**c**) RT-PCR of gene expression by CD4$^+$ T cells purified from draining lymph nodes of wild-type mice (black bars), IL-23p19-deficient mice (dark gray bars) and IL-12p35-deficient mice (light gray bars) vaccinated as described in **a**. Data are expressed as 'fold induction' relative to day 0 (**b**) or unvaccinated (Un; **c**). (**d**,**e**) Frequency (**d**) and total number (**e**) of cells that divided in the draining lymph nodes of wild-type mice (black bars), IL-23p19-deficient mice (dark gray bars) and IL-12p35-deficient mice (light gray bars) that received naive CFSE-labeled OT-II CD4$^+$ T cells and were then vaccinated with OVA(323–339) in MPL plus TDM plus DDA, assessed by CFSE dilution in transferred cells by day 3. (**f**,**g**) ELISPOT analysis of the frequency (**f**,**g**) and total number (**Supplementary Fig. 1**) of IL-17-producing cells (**f**) or IFN-γ-producing cells (**g**) in various organs of wild-type mice (black bars), IL-23p19-deficient mice (dark gray bars) and IL-12p35-deficient mice (light gray bars) vaccinated as described in **a**, assessed 30 d after vaccination. DLN, draining lymph node. *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$, and ****, $P \leq 0.0001$, versus day 0 (**b**), unvaccinated (**c**) or wild-type (**e**–**g**; Student's *t*-test). Data (mean + s.d.) are one representative of two independent experiments with four (**a**,**d**,**e**) or four to six (**b**,**c**,**f**,**g**) mice.

vaccinated mice was dependent on IL-23 and IL-17 and that IL-17 alone was able to restore the IFN-γ recall response in vaccinated IL-23p19-deficient mice.

Cytokines in vaccination-induced T cell responses

To investigate how IL-23 affects T cell activation after vaccination, we tracked the formation of antigen-specific IL-17-producing CD4$^+$ T cells in wild-type and cytokine-deficient mice after vaccination. Wild-type and IL-12p35-deficient mice showed a measurable and sustained IL-17 response after vaccination, whereas IL-23p19-deficient mice showed an early but short-lived antigen-specific IL-17 T cell response (**Fig. 6a**). To determine whether IL-23 acted during the priming of IL-17-producing cells, we analyzed the kinetics of expression of IL-23 and its receptor after vaccination. For this, we vaccinated mice expressing yellow fluorescent protein (YFP) in the locus encoding IL-12p40 (B6.yet40 mice; ref. 39) and compared IL-12p40-expressing YFP$^+$CD11c$^+$ cells from the draining lymph nodes of vaccinated and naive mice. Transcripts encoding IL-23p19 (*Il23a*) were expressed early (day 4) but not late, whereas the gene encoding IL-12p35 (*Il12a*) was expressed by day 17 (**Fig. 6b**). YFP$^+$CD11c$^+$ cells also had significant early induction of transcripts encoding TGF-β (*Tgfb1*) and IL-6 (*Il6*) (**Fig. 6b**), both of which have been linked to the differentiation of T_H-17 cells[16–18]. Although we found no change in the expression of IL-12 and IL-23 receptors in CD4$^+$ T cells from draining lymph nodes of vaccinated mice relative to unvaccinated mice on days 2 and 4 (data not shown), we detected significant induction of transcripts encoding the IL-23 receptor (*Il23r*) but not IL-12 receptor-β2 (*Il12rb2*) by day 7 (**Fig. 6c**). At day 7, CD4$^+$ T cells did not yet express *Ifng* transcripts, but had substantial expression of *Il17a* mRNA in an IL-23-independent way (**Fig. 6c**). These data suggested that both IL-23 and its receptor were present on CD4$^+$ T cells rapidly after vaccination but that IL-23 was dispensable for inducing the differentiation of T_H-17 cells.

To determine whether IL-23 influenced the initial proliferation of naive antigen-specific CD4$^+$ T cells after vaccination, we labeled naive CD4$^+$ T cells expressing the OT-II TCR transgene, which recognizes the peptide antigen comprising ovalbumin amino acids 323–339 (OVA(323–339)), with CSFE (5-(and-6)-carboxyfluorescein

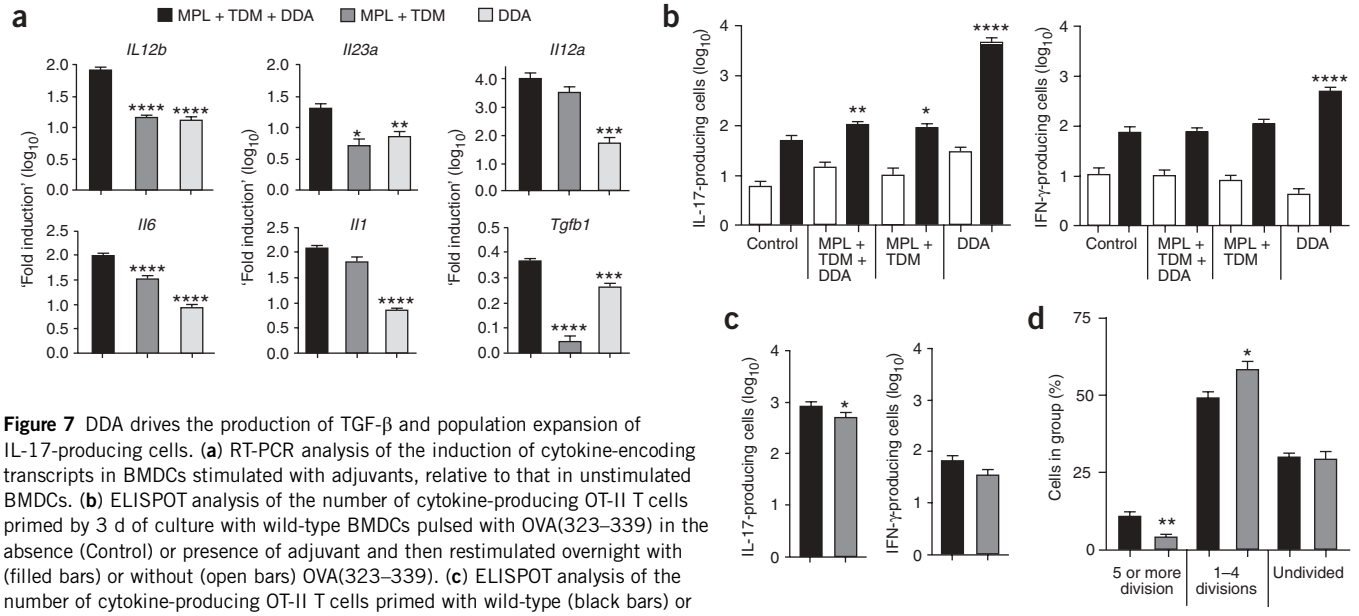

Figure 7 DDA drives the production of TGF-β and population expansion of IL-17-producing cells. (**a**) RT-PCR analysis of the induction of cytokine-encoding transcripts in BMDCs stimulated with adjuvants, relative to that in unstimulated BMDCs. (**b**) ELISPOT analysis of the number of cytokine-producing OT-II T cells primed by 3 d of culture with wild-type BMDCs pulsed with OVA(323–339) in the absence (Control) or presence of adjuvant and then restimulated overnight with (filled bars) or without (open bars) OVA(323–339). (**c**) ELISPOT analysis of the number of cytokine-producing OT-II T cells primed with wild-type (black bars) or IL-23p19-deficient (dark gray bars) BMDCs stimulated with MPL plus TDM plus DDA and OVA(323–339) and then restimulated overnight with OVA(323–339). (**d**) Flow cytometry of the frequency of cells having undergone division among CFSE-labeled naive OT-II T cells cultured for 3 d with OVA(323–339)-pulsed wild-type BMDCs (black bars) or IL-23p19-deficient BMDCs (dark gray bars) that were stimulated with MPL plus TDM plus DDA. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, and ****, $P < 0.0001$, relative to MPL plus TDM plus DDA (**a**), no adjuvant (**b**) or wild-type (**c**,**d**; Student's *t*-test). Data (mean + s.d.) are one representative of two independent experiments with three (**a**–**c**) or four to six (**d**) separate cultures.

succinimidyl ester). We then transferred the CFSE-labeled cells into wild-type or cytokine-deficient mice. Vaccination with OVA (323–339) in adjuvant triggered the proliferation of naive OT-II $CD4^+$ T cells in wild-type and IL-12p35-deficient mice, but IL-23p19-deficient mice had less proliferation (**Fig. 6d**,**e**).

To determine whether the absence of IL-23 affected the generation of long-lived cells after vaccination, we determined the frequency (**Fig. 6f**,**g**) and number (**Supplementary Fig. 2** online) of antigen-specific $CD4^+$ T cells in the central and peripheral tissues of mice 30 d after vaccination. We detected a substantial population of antigen-specific IL-17-producing $CD4^+$ T cells in the lungs of vaccinated wild-type and IL-12p35-deficient but not IL-23p19-deficient mice (**Fig. 6f** and **Supplementary Fig. 2**), until at least 105 d after vaccination (data not shown). In contrast, few antigen-specific IFN-γ-producing $CD4^+$ T cells were present in the lung (**Fig. 6g**). We found substantial populations of vaccination-induced IFN-γ- and IL-17-producing $CD4^+$ T cells in the spleens and draining lymph nodes. In all organs, the number of IL-17- but not IFN-γ-producing cells depended on the presence of IL-23. Antigen-specific T cells present in the lung after vaccination had high surface expression of CD44 and the tissue-homing chemokine receptor CCR4 (**Supplementary Fig. 2**). These data demonstrated the requirement for IL-23 for the establishment of a T_H-17 memory population after vaccination.

To define the component of the vaccine responsible for the induction of IL-17-producing cells, we cultured bone marrow–derived DCs (BMDCs) with various adjuvant components and measured their expression of transcripts encoding various cytokines and their ability to polarize OT-II T cells. MPL plus TDM promoted significantly greater induction of mRNA encoding IL-12p35 and IL-1 than did DDA alone, whereas the addition of DDA to MPL plus TDM promoted significant upregulation of transcripts encoding IL-12p40, IL-6 and IL-23p19 relative to MPL plus TDM alone (**Fig. 7a**). In contrast, DDA triggered higher expression of *Tgfb1*; this induction was not lost when MPL, TDM and DDA were added together (**Fig. 7a**). These adjuvant components also stimulated antigen-pulsed BMDCs to prime OT-II T cells producing IL-17 and OT-II T cells producing IFN-γ; however, DDA alone was most potent in eliciting cytokine-producing cells (**Fig. 7b**). This ability was minimally dependent on IL-23 (**Fig. 7c**), consistent with the observation that the presence of IL-23 did not affect the amount of vaccine-induced *Il17a* in $CD4^+$ T cells in the draining lymph nodes (**Fig. 6c**). However, absence of IL-23 reduced the ability of naive OT-II $CD4^+$ T cells to progress through multiple cell divisions *in vitro* (**Fig. 7d**). That observation supported *in vivo* data showing that division of naive T cells after vaccination was lower in the absence of IL-23 (**Fig. 6d**). These data collectively demonstrated a function for IL-23 in promoting optimal proliferation of newly activated T cells but not in polarizing naive T cells toward the T_H-17 lineage. We propose a model for the potential pathway by which vaccine-induced protection depends on IL-23 and IL-17 (**Supplementary Fig. 3** online).

DISCUSSION

Here, a population of IL-23-dependent, IL-17-producing, antigen-specific T cells was induced in response to vaccination and populated the lung. The recall response of this population preceded the IFN-γ recall response in the lungs and occurred simultaneously with induction of the CXCR3 ligand chemokines CXCL9, CXCL10 and CXCL11. In the absence of IL-23 or IL-17, there was less chemokine expression and a lower IFN-γ recall response in vaccinated mice, whereas IL-17 restored the IFN-γ recall response in the absence of IL-23. These data demonstrate a previously unappreciated function for IL-23 and IL-17 in vaccination and support a new model for understanding $CD4^+$ T cell recall responses to lung infections. Specifically, IL-17-producing $CD4^+$ T cells provide a surveillance function in peripheral tissues, such that when antigen is detected, they respond rapidly and recruit other effector recall responses, which then stop pathogen growth.

Our data alter the understanding of the relative functions of IL-12 and IL-23 in vaccine-induced, long-lived, antigen-specific T cell responses. Specifically, an IFN-γ recall response to challenge was triggered, albeit to a lower degree, in the absence of IL-12. In contrast, whereas IL-23 was not required for the initiation of IFN-γ-producing $CD4^+$ T cells after vaccination, in its absence, IL-17 as well as IFN-γ recall responses were impaired. Our results shed new light on published studies using IL-12p40-deficient mice and antibodies specific for IL-12p40, which indicated involvement of IL-12 but were done before IL-23 was identified[12,13]. It is likely that those data were a result of inhibition of both IL-12 and IL-23 rather than of IL-12 alone. In contrast, however, other published data have demonstrated that protection mediated by DNA vaccination is restored by either IL-23 or IL-12, suggesting that when cells other than $CD4^+$ T cells are induced by vaccination, the dependence on IL-23 is not absolute[40].

IL-23 seemed to act during vaccination rather than challenge, as IL-17-producing cells generated in the presence of IL-23 persisted as a peripheral population and promoted both a recall response and protection against bacterial challenge. However, priming of antigen-specific IL-17-producing cells occurred in the absence of IL-23 both *in vivo* and *in vitro*, but this response was not maintained *in vivo* and was compromised over time *in vitro*[14,15]. This is in agreement with published studies demonstrating a need for IL-23 in the proliferation and persistence but not the induction of IL-17-producing cells[20,28,29,32,41]. However, our data have shown that IL-23 was expressed early in the draining lymph nodes after vaccination and at the same time as the canonical IL-17-inducing cytokines TGF-β and IL-6. Furthermore, $CD4^+$ T cells from the draining lymph nodes expressed IL-23 receptor but not IL-12 receptor-β2 early, and the absence of IL-23 affected the proliferation of newly activated cells both *in vivo* and *in vitro*. As IL-23 can act directly on DCs[42], we cannot distinguish between a direct effect on T cells or an indirect effect through defective DC function; this remains to be investigated. These data suggest that IL-23 acts early during priming but that its absence has consequences in terms of persistence of the antigen-specific IL-17-producing population rather than priming itself.

Several pathogens promote the differentiation of IL-17-producing cells *in vivo*[14,15,43], suggesting that such cells can be induced by a variety of stimuli. We have shown here that the small cationic molecule DDA drove the polarization of naive T cells to an IL-17-producing phenotype *in vitro*. We presume that DDA acted by promoting TGF-β and IL-6 expression in BMDCs and that these cytokines 'provoke' naive T cells to become IL-17 producers rather than regulatory T cells[16,18]. The relative functions of DDA, MPL and TDM in driving the development of persistent peripheral IL-17-producing cells *in vivo* remain to be determined.

Notably, IL-17-producing cells induced by vaccination populated the lung, whereas IFN-γ-producing cells remained in the central lymphoid tissue. The lung cells seemed to be activated, as they expressed CD44. They also expressed CCR4, which has been associated with the ability of cells to traffic into peripheral tissues[44]. This suggests that IL-17-producing cells either 'preferentially' trafficked through or resided in the lungs. Regardless of which is true, we hypothesize that IL-17-producing cells responded directly in the lung, as we detected ESAT-6(1–20)-specific IL-17-producing cells earlier in the lung than in the draining lymph nodes after challenge in vaccinated mice (data not shown). Other studies have also shown that previously activated $CD4^+$ T cells can encounter antigen in the lung before the lymph node[45]. This 'preference' for the periphery and the rapid response of these cells makes them candidate immune surveillance cells.

Although we have proposed new functions for IL-23 and IL-17, it was the arrival of IFN-γ-producing cells, not the IL-17-producing cells, in the lung that correlated with the expression of antibacterial molecules and the cessation of bacterial growth in this tissue. However, as the absence of an IL-17 recall response precluded the IFN-γ recall response and the accumulation of $CXCR3^+CD44^+CD4^+$ T cells, it seems that the IL-17-dependent chemokine response was indirectly essential for the accelerated antimycobacterial activity. The ability of IL-17 to mediate cellular infiltration through chemokine induction has been reported for neutrophils[46] and macrophages[47], but its ability to promote T cell accumulation is not widely accepted. These data demonstrating the ability of anti-IL-17 to block chemokine induction and the presence of transcription factor–binding patterns associated with IL-17-responsive genes in the promoter regions of genes encoding these chemokines suggest direct induction by IL-17. Alternatively, IL-17 could promote the recruitment of neutrophils, which might produce the chemokines, as suggested before[48]. The mechanism whereby IL-17 promotes the induction of chemokines remains to be determined.

It has been shown that IL-17-producing cells are induced by vaccination and that treatment with anti-IL-17 reduces protection from pulmonary challenge with *Bordetella pertussis*[43]. The function of IL-17 in promoting recruitment of the IFN-γ recall response was not assessed in that model, and IL-17 was thought to be acting through macrophage activation, based on *in vitro* data[43]. We believe that a function for IL-17 in activating macrophages in our model is unlikely, as we found no sign of macrophage activation until IFN-γ-producing cells arrived in the lung. Other studies have failed to identify a function for IL-23 or IL-17 in a bacille Calmette-Guerin vaccine model with challenge administered intravenously[49]; that probably reflects a limited need for a surveillance population when recall responses are rapid and systemic[4].

In summary, we propose that IL-17-producing cells induced by vaccination can provide a surveillance function in the periphery. This is important, as greater numbers of these cells may promote more rapid responses to pulmonary challenge. However, peripherally located IL-17-producing cells are known to have a destructive function[20,22,50], and balancing the positive and negative activities of these cells to use them as mediators of vaccine-induced protection will thus require thorough understanding of their biology.

METHODS

Mice. C57BL/6J mice, C57BL/6J CD90.1 congenic mice (B6.PL-*Thy1*^{a}/Cy), IL-12p35-deficient mice (B6.129S1-*Il12a*^{tm1Jm}/J), IL-12p40 deficient mice (B6.129S1-*Il12b*^{tm1Jm}/J) and IFN-γ-deficient mice (B6.129S7-*Ifng*^{tm1Ts}/J) were purchased from The Jackson Laboratory. IL-23p19-deficient mice (B6.*Il23a*$^{-/-}$) have been described[51]. B6.yet40 mice were generated as described[39]. OT-II TCR–transgenic male mice have been described[52]. Mice between 8 and 12 weeks of age were used; experimental mice were age and sex matched. All studies were approved by the Trudeau Institute Institutional Animal Care and Use Committee.

Immunization. Epitope-specific protective $CD4^+$ T cells were induced with the immunodominant I-A^b-restricted ESAT-6(1–20) epitope[33] (New England Peptide). This peptide was mixed with DDA (Eastman Kodak) and then was emulsified with the adjuvant MPL plus TDM (Sigma-Aldrich) as described[53]. MPL plus TDM is a stable oil-in-water emulsion containing inflammatory, nontoxic MPL and the trehalose dimycolate analog TDM. Mice were injected once subcutaneously with 400 μg peptide in 0.2 ml adjuvant. Some mice received the I-A^b-restricted epitope OVA(323–339).

Infection, challenge and cytokine and antibody treatment. The H37Rv strain of *M. tuberculosis* was used to infect mice by the aerosol route, as described[14].

Some mice received recombinant IL-17A (R&D Systems) intratracheally at a dose of 1.5 μg in 50 μl saline per mouse[54]; this product had less than 0.1 ng endotoxin per microgram of cytokine (as determined by manufacturer). Some mice received 100 μg of isotype control antibody (50104) or anti-IL-17 (54447; both from R&D Systems) intraperitoneally on days 12 and 14 after infection. Bacteria in the lungs were measured by counting of viable colony-forming units in homogenized tissue as described[14].

Cell preparation and culture. Lung, lymph node and spleen cell suspensions were prepared as described[14]. Cells were used for enzyme-linked immunospot (ELISPOT) assay or flow cytometry or were fractionated based on CD4 expression. $CD4^+$ T cells were isolated from cell suspensions with magnetic $CD4^+$ beads (GK1.5; Miltenyi Biotech) on an AutoMACS machine according to the manufacturer's instructions. Naive $CD4^+$ T cells from OT-II mice were 'loaded' with CFSE as described, and 1×10^6 cells were transferred intravenously into wild-type or cytokine-deficient host mice[52]. Mice receiving CFSE-loaded naive T cells were then vaccinated and the frequency of divided cells in the draining lymph nodes was determined by flow cytometry.

***In vitro* stimulation of naive T cells and BMDCs.** BMDCs were generated as described[14] and then were cultured with 10 μl of vaccine preparation components; RNA was extracted with the RNeasy kit (Qiagen) from stimulated BMDCs over time. Adjuvant-stimulated BMDCs were also pulsed with cognate antigen and were used to drive the activation for 3 d of naive $CD4^+$ T cells purified from OT-II mice. ELISPOT assays were used to measure cytokine production. Cells were labeled with CFSE and loss of CFSE was measured by flow cytometry.

ELISPOT assay. Antigen-specific IFN-γ- and IL-17-producing cells in infected lungs were detected by ELISPOT assay as described[14]. Cells were seeded in antibody-coated plates at an initial density of 5×10^5 or 1×10^6 cells per well. Irradiated wild-type splenocytes, 10 μg/ml of ESAT-6(1–20) or OVA(323–339) peptide, and 10 U/ml of mouse recombinant IL-2 (Sigma) were added to each well. After 18 h, plates were developed and the frequency and total number of responding cells was determined. Neither cells cultured in the absence of peptide nor cells from uninfected mice produced detectable spots.

Real-time PCR. RNA was extracted from total lung tissue or $CD4^+$ separated cells with the RNeasy Kit (Qiagen) as recommended by the manufacturer. RNA samples were treated with DNase and reverse-transcribed and cDNA was amplified with Taqman reagents on the ABI Prism 7700 sequence detection system (Applied Biosystems)[14]. Samples were prepared without reverse-transcription enzyme to confirm that signal was derived from RNA. The 'fold increase' in signal relative to that of uninfected or unvaccinated samples was determined with the '$\Delta\Delta C_T$' (change in cycling threshold) calculation recommended by Applied Biosystems. The primer and probe sequences for mouse *Ifng*, *Irgm* (encoding LRG-47), *Tnf*, *Il17a* and *Cxcl10* have been published[14,55]. Primers and probes for *Cxcl9* (mm0434946) and *Ccl11* (mm0044662) were purchased from Applied Biosystems Gene Expression Assays.

Flow cytometry. Single-cell suspensions from the lungs or draining lymph nodes were prepared as described above and cells were stained with labeled antibodies specific for CD11c (HL3), $I\text{-}A^b$ (AF6-120.1), CD4 (GK1.5), CD3 (145-2C11; all from Becton Dickinson), CCR4 (205410; Caprologics), CXCR3 (220803; R&D Systems) or CD44 (IM7; eBiosciences). Data were collected with CellQuest software on a FACSCalibur (Becton Dickinson). DCs were identified based on forward scatter, CD11c expression and low autofluorescence[52]. $CD11c^+$ yellow fluorescent cells from the draining lymph nodes of both vaccinated and unvaccinated B6.yet40 mice were sorted with a FACSVantage SE with the DIVA option (Becton Dickinson) as described[56].

Morphometric analysis and immunohistochemistry. Caudal lobes of lungs from vaccinated and naive mice were inflated with 10% (vol/vol) formalin in buffered saline, were fixed for 72 h and were embedded in paraffin. Sections 5 μm in thickness were stained with hematoxylin and eosin. Caudal lobes from four mice per group were analyzed by researchers 'blinded' to sample identify using the morphometric tool of the Zeiss Axioplan microscope (Zeiss). This tool determines the area defined by the squared pixel value for each granuloma. Paraffin was removed from the formalin-fixed lung sections, which were then washed with xylene, alcohol and PBS. Antigens were 'unmasked' with DakoCytomation Target Retrieval Solution (Dako) and were blocked with 5% (vol/vol) normal donkey serum and Fc block (5 μg/ml; 2.4G27; Trudeau Institute). Endogenous biotin was neutralized with avidin followed by biotin (both from Sigma Aldrich). Sections were probed with goat anti-mouse specific for inducible nitric oxide synthase (M-19.G; Santa Cruz Biotechnology), which was detected with Alexa Fluor 594–conjugated polyclonal donkey anti-goat (Molecular Probes). DAPI (4′,6-diamido-2-phenylindole hydrochloride) was used to counterstain tissues and to detect nuclei. Pictures were obtained with a Zeiss Axioplan 2 microscope. Images were recorded with a Zeiss AxioCam digital camera.

***In vitro* population expansion of ESAT-6-specific T cells.** Lymphocytes were isolated from draining lymph nodes and spleens of B6 CD90.1 congenic mice vaccinated 10 d before with ESAT-6(1–20) in MPL plus TDM plus DDA. Cells were cultured for 6 d *in vitro* with wild-type APCs, 10 U/ml of IL-2, 10 μg/ml of ESAT-6(1–20), 10 μg/ml of anti-IL-4 (clone 11B11), 10 μg/ml of anti-IFN-γ (clone XMG1.2) and 50 ng/ml of recombinant IL-23 (R&D Systems). Cells were then analyzed for their ability to produce IFN-γ or IL-17 after 5 h of culture with 50 ng/ml of phorbol 12-myristate 13-acetate and 750 ng/ml of ionomycin (both from Sigma). Cytokines were measured by intracellular staining as described[14]. $CD4^+$ T cells were purified from cultures as described above, and 1×10^6 cells were transferred intravenously into host mice.

Statistical analysis. Differences between the means of experimental groups were analyzed with the two-tailed Student's *t*-test. Differences with a *P* value of 0.05 or less were considered significant. Inherently logarithmic data from bacterial growth, cell proliferation and RT-PCR were transformed for statistical analysis.

Note: Supplementary information is available on the Nature Immunology website.

ACKNOWLEDGMENTS

We thank S. Smiley, L. Johnson, M. Mohrs, M. Blackman, R. Dutton, G. Winslow and D. Woodland for critical reading of the manuscript, and B. Sells for cell sorting. IL-23p19-deficient mice (B6.*Il23a*$^{-/-}$) were provided by N. Ghilardi and F. deSauvage (Genentech). Supported by the Trudeau Institute, the New York Community Trust–Heiser Fund (S.A.K.) and the National Institutes of Health (AI46530, AI067723 and AG028878 to A.M.C.; AR050458 to S.L.G.; AI030663 to R.M.L.; AG02160 and AG21054 to L.H.; and HL69409 to T.D.R.).

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

Published online at http://www.nature.com/natureimmunology/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Corbett, E.L. *et al.* The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* **163**, 1009–1021 (2003).
2. Nabeshima, S. *et al.* Kinetic analysis of *Mycobacterium tuberculosis*-specific cytokine production by PBMC in adults after BCG vaccination. *J. Infect. Chemother.* **11**, 18–23 (2005).
3. Colditz, G.A. *et al.* Efficacy of BCG vaccine in the prevention of tuberculosis. *J. Am. Med. Assoc.* **271**, 698–702 (1994).
4. Cooper, A.M., Callahan, J.E., Keen, M., Belisle, J.T. & Orme, I.M. Expression of memory immunity in the lung following re-exposure to *Mycobacterium tuberculosis*. *Tuber. Lung Dis.* **78**, 67–73 (1997).
5. Jung, Y.J., Ryan, L., Lacourse, R. & North, R. Properties and protective value of the secondary versus primary T helper type 1 response to airborne *Mycobacterium tuberculosis* infection in mice. *J. Exp. Med.* **201**, 1915–1924 (2005).
6. Flynn, J.L. & Chan, J. Immunology of tuberculosis. *Annu. Rev. Immunol.* **19**, 93–129 (2001).
7. Akahoshi, M. *et al.* Influence of interleukin-12 receptor β1 polymorphisms on tuberculosis. *Hum. Genet.* **112**, 237–243 (2003).
8. Tso, H.W., Lau, Y.L., Tam, C.M., Wong, H.S. & Chiang, A.K. Associations between IL12β polymorphisms and tuberculosis in the Hong Kong chinese population. *J. Infect. Dis.* **190**, 913–919 (2004).
9. Cooper, A.M., Magram, J., Ferrante, J. & Orme, I.M. IL-12 is crucial to the development of protective immunity in mice intravenously infected with *Mycobacterium tuberculosis*. *J. Exp. Med.* **186**, 39–46 (1997).
10. Holscher, C. *et al.* A protective and agonistic function of IL-12p40 in mycobacterial infection. *J. Immunol.* **167**, 6957–6966 (2001).

11. Cooper, A.M. *et al.* Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. *J. Immunol.* **168**, 1322–1327 (2002).
12. Leal, I.S., Smedegard, B., Andersen, P. & Appelberg, R. Interleukin-6 and interleukin-12 participate in induction of a type 1 protective T-cell response during vaccination with a tuberculosis subunit vaccine. *Infect. Immun.* **67**, 5747–5754 (1999).
13. Stobie, L. *et al.* The role of antigen and IL-12 in sustaining Th1 memory cells *in vivo*: IL-12 is required to maintain memory/effector Th1 cells sufficient to mediate protection to an infectious parasite challenge. *Proc. Natl. Acad. Sci. USA* **97**, 8427–8432 (2000).
14. Khader, S.A. *et al.* IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-γ responses if IL-12p70 is available. *J. Immunol.* **175**, 788–795 (2005).
15. Cruz, A. *et al.* CE:IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection1. *J. Immunol.* **177**, 1416–1420 (2006).
16. Veldhoen, M., Hocking, R., Atkins, C., Locksley, R. & Stockinger, B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* **24**, 179–189 (2006).
17. Mangan, P.R. *et al.* Transforming growth factor-β induces development of the T_H17 lineage. *Nature* **441**, 231–234 (2006).
18. Bettelli, E. *et al.* Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. *Nature* **441**, 235–238 (2006).
19. Aggarwal, S. & Gurney, A.L. IL-17: prototype member of an emerging cytokine family. *J. Leukoc. Biol.* **71**, 1–8 (2002).
20. Langrish, C.L. *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201**, 233–240 (2005).
21. Nakae, S., Nambu, A., Sudo, K. & Iwakura, Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* **171**, 6173–6177 (2003).
22. Nakae, S. *et al.* IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc. Natl. Acad. Sci. USA* **100**, 5986–5990 (2003).
23. Fossiez, F. *et al.* T cell interleukin-17 induces stromal cells to produce pro-inflammatory and hematopoietic cytokines. *J. Exp. Med.* **183**, 2593–2603 (1996).
24. Jones, C.E. & Chan, K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-α, and granulocyte-colony-stimulating factor by human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **26**, 748–753 (2002).
25. Ye, P. *et al.* Requirement of Interleukin-17 receptor signalling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recriutment, and host defense. *J. Exp. Med.* **194**, 519–527 (2001).
26. Kolls, J.K., Kanaly, S.T. & Ramsay, A.J. Interleukin-17: An emerging role in lung inflammation. *Am. J. Respir. Cell Mol. Biol.* **28**, 9–11 (2003).
27. Moseley, T.A., Haudenschild, D., Rose, L. & Reddi, A. Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev.* **14**, 155–174 (2003).
28. Park, H. *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**, 1133–1141 (2005).
29. Harrington, L.E. *et al.* Interleukin 17–producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).
30. Oppmann, B. *et al.* Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**, 715–725 (2000).
31. Parham, C. *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J. Immunol.* **168**, 5699–5708 (2002).
32. Aggarwal, S., Ghilardi, N., Xie, M-H., deSauvage, F. & Gurney, A. Interleukin 23 promotes a distinct CD4 T cell activation state characterized by the production of IL-17. *J. Biol. Chem.* **278**, 1910–1914 (2003).
33. Winslow, G.M., Roberts, A.D., Blackman, M.A. & Woodland, D.L. Persistence and turnover of antigen-specific CD4 T cells during chronic tuberculosis infection in the mouse. *J. Immunol.* **170**, 2046–2052 (2003).
34. Evans, J. *et al.* Enhancement of antigen-specific immunity via the TLR4 ligands MPL adjuvant and Ribi.529. *Expert Rev. Vaccines* **2**, 219–229 (2003).
35. Geisel, R.E., Sakamoto, K., Russell, D. & Rhoades, E. *In vivo* activity of released cell wall lipids of *Mycobacterium bovis* bacillus Calmette-Guerin is due principally to trehalose mycolates. *J. Immunol.* **174**, 5007–5015 (2005).
36. Kim, C.H. & Broxmeyer, H.E. Chemokines: signal lamps for trafficking of T and B cells for development and effector function. *J. Leukoc. Biol.* **65**, 6–15 (1999).
37. MacMicking, J.D., Taylor, G. & McKinney, J. Immune control of tuberculosis by IFN-γ-inducible LRG-47. *Science* **302**, 654–659 (2003).
38. Shen, F., Hu, Z., Goswami, J. & Gaffen, S. Identification of common transcriptional regulatory elements in interleukin-17 target genes. *J. Biol. Chem.* **281**, 24138–24148 (2006).
39. Reinhardt, R.L., Hong, S., Kang, S., Wang, Z. & Locksley, R. Visualization of IL-12/23p40 *in vivo* reveals immunostimulatory dendritic cell migrants that promote Th1 differentiation. *J. Immunol.* **177**, 1618–1627 (2006).
40. Wozniak, T.M., Ryan, A. & Britton, W. Interleukin-23 restores immunity to *Mycobacterium tuberculosis* infection in IL-12p40-deficient mice and is not required for the development of IL-17-secreting T cell responses. *J. Immunol.* **177**, 8684–8692 (2006).
41. Veldhoen, M., Hocking, R., Flavell, R. & Stockinger, B. Signals mediated by transforming growth factor-β initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat. Immunol.* **7**, 1151–1156 (2006).
42. Belladonna, M.L. *et al.* IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J. Immunol.* **168**, 5448–5454 (2002).
43. Higgins, S.C., Jarnicki, A., Lavelle, E. & Mills, K. TLR4 Mediates vaccine-Induced protective cellular immunity to *Bordetella pertussis*: role of IL-17-producing T cells. *J. Immunol.* **177**, 7980–7989 (2006).
44. Baekkevold, E.S. *et al.* A role for CCR4 in development of mature circulating cutaneous T helper memory cell populations. *J. Exp. Med.* **201**, 1045–1051 (2005).
45. Byersdorfer, C.A. & Chaplin, D. Visualization of early APC/T cell interactions in the mouse lung following intranasal challenge. *J. Immunol.* **167**, 6756–6764 (2001).
46. Kolls, J.K. & Linden, A. Interleukin-17 family members and inflammation. *Immunity* **21**, 467–476 (2004).
47. Miyamoto, M. *et al.* Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. *J. Immunol.* **170**, 4665–4672 (2003).
48. Appelberg, R. Neutrophils and intracellular pathogens: beyond phagocytosis and killing. *Trends Microbiol.* **15**, 87–92 (2007).
49. Chackerian, A.A. *et al.* Neutralization or absence of the interleukin-23 pathway does not compromise immunity to mycobacterial infection. *Infect. Immun.* **74**, 6092–6099 (2006).
50. Gaffen, S.L. Biology of recently discovered cytokines: interleukin-17–a unique inflammatory cytokine with roles in bone biology and arthritis. *Arthritis Res. Ther.* **6**, 240–247 (2004).
51. Ghilardi, N. *et al.* Compromised humoral and delayed-type hypersensitivity responses in IL-23-deficient mice. *J. Immunol.* **172**, 2827–2833 (2004).
52. Khader, S.A. *et al.* Interleukin 12p40 is required for dendritic cell migration and T cell priming after *Mycobacterium tuberculosis* infection. *J. Exp. Med.* **203**, 1805–1815 (2006).
53. Olsen, A.W., Hansen, P., Holm, A. & Andersen, P. Efficient protection against *Mycobacterium tuberculosis* by vaccination with a single immunodominant epitope from the ESAT-6 antigen. *Eur. J. Immunol.* **30**, 1724–1732 (2000).
54. Happel, K.I. *et al.* Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J. Exp. Med.* **202**, 761–769 (2005).
55. Hollenbaugh, J.A. & Dutton, R. IFN-γ regulates donor CD8 T cell expansion, migration and leads to solid tumor apoptosis. *J. Immunol.* **177**, 3004–3011 (2006).
56. Flano, E., Kayhan, B., Woodland, D. & Blackman, M. Infection of dendritic cells by a γ2-herpesvirus induces functional modulation. *J. Immunol.* **175**, 3225–3234 (2005).