LETTER

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Pathogen-induced human T_H17 cells produce IFN-γ or IL-10 and are regulated by IL-1β

Christina E. Zielinski[1]†, Federico Mele[1], Dominik Aschenbrenner[1], David Jarrossay[1], Francesca Ronchi[1], Marco Gattorno[2], Silvia Monticelli[1], Antonio Lanzavecchia[1,3] & Federica Sallusto[1]

IL-17-producing $CD4^+$ T helper cells (T_H17) have been extensively investigated in mouse models of autoimmunity[1]. However, the requirements for differentiation and the properties of pathogen-induced human T_H17 cells remain poorly defined. Using an approach that combines the *in vitro* priming of naive T cells with the *ex vivo* analysis of memory T cells, we describe here two types of human T_H17 cells with distinct effector function and differentiation requirements. *Candida albicans*-specific T_H17 cells produced IL-17 and IFN-γ, but no IL-10, whereas *Staphylococcus aureus*-specific T_H17 cells produced IL-17 and could produce IL-10 upon restimulation. IL-6, IL-23 and IL-1β contributed to T_H17 differentiation induced by both pathogens, but IL-1β was essential in *C. albicans*-induced T_H17 differentiation to counteract the inhibitory activity of IL-12 and to prime IL-17/IFN-γ double-producing cells. In addition, IL-1β inhibited IL-10 production in differentiating and in memory T_H17 cells, whereas blockade of IL-1β *in vivo* led to increased IL-10 production by memory T_H17 cells. We also show that, after restimulation, T_H17 cells transiently downregulated IL-17 production through a mechanism that involved IL-2-induced activation of STAT5 and decreased expression of ROR-γt. Taken together these findings demonstrate that by eliciting different cytokines *C. albicans* and *S. aureus* prime T_H17 cells that produce either IFN-γ or IL-10, and identify IL-1β and IL-2 as pro- and anti-inflammatory regulators of T_H17 cells both at priming and in the effector phase.

T_H17 cells participate in host defence against fungi and extracellular bacteria[2]. Patients with genetic defects in the T_H17 axis suffer from recurrent infections with *Candida albicans* and *Staphylococcus aureus*[3–5]. Understanding the regulation of T_H17 differentiation induced by these pathogens is therefore of both fundamental and clinical relevance.

The requirements for T_H17 differentiation in humans have been studied using polyclonal activators and recombinant cytokines. Here, we developed an antigen-specific T-cell-priming approach using whole microbes and monocytes as antigen-presenting cells. This approach takes advantage of the complexity of the microbes that provide, at the same time, a large number of antigens and a variety of stimuli for innate receptors to elicit polarizing cytokines. When carboxyfluorescein succinimidyl ester (CFSE)-labelled human naive $CD4^+$ T cells were cultured with autologous monocytes pulsed with *C. albicans* or *S. aureus*, antigen-specific proliferating T cells could be detected after 12 days in microbe-stimulated cultures, but not in cultures performed in the presence of antibodies blocking major histocompatibility complex (MHC) class II molecules (Supplementary Fig. 1a, b). In both *C. albicans*- and *S. aureus*-primed cultures a substantial fraction of proliferating cells acquired the capacity to produce IL-17 and IL-22, and expressed ROR-γt and CCR6, which are characteristics of T_H17 cells[6,7] (Fig. 1a and Supplementary Fig. 1c). Interestingly, in *C. albicans*-primed cultures most IL-17-secreting cells also produced IFN-γ and expressed ROR-γt and T-bet, whereas in *S. aureus*-primed cultures most IL-17-secreting cells did not produce IFN-γ and did not express T-bet (Fig. 1b–d). Both cultures also contained T cells that produced IFN-γ only, whereas very few cells produced IL-10. These findings demonstrate that primary T-cell responses to *C. albicans* and *S. aureus* can be generated *in vitro*, leading to different types of polarized T cells: IL-17 single-producers, IL-17/IFN-γ double-producers (hereafter defined collectively as T_H17) and IFN-γ single-producers (hereafter defined as T_H1).

To investigate whether and under which conditions microbe-specific T_H17 cells produce IL-10 (ref. 8–13), we isolated T_H17 clones from *in-vitro*-primed cultures and measured their cytokine production capacity in the resting state and at different time points after restimulation (Fig. 1e, f). Resting T_H17 clones produced high amounts of IL-17 but virtually no IL-10. Surprisingly, when tested on day 5 after restimulation, *S. aureus*-specific but not *C. albicans*-specific T_H17 clones acquired IL-10 production capacity. In addition, both *S. aureus*- and *C. albicans*-specific T_H17 clones strongly downregulated IL-17 production. At later time points all clones gradually regained the original cytokine profile as the cells reverted to the resting state. These results reveal a reciprocal activation-dependent regulation of IL-17 and IL-10 production in human T_H17 cells.

The above findings show that *C. albicans* and *S. aureus* prime *in vitro* T_H17 cells with different capacities to produce IFN-γ and IL-10. To investigate whether these properties are also characteristic of *in-vivo*-primed T cells, we isolated memory T cell subsets according to the expression of chemokine receptors (Supplementary Fig. 2) and stimulated them with *C. albicans*- or *S. aureus*-pulsed autologous monocytes. In all donors tested, a robust proliferative response to *C. albicans* and *S. aureus* was detected in the $CCR6^+CCR4^+$ subset, which contains T_H17 as well as some T_H1 cells, whereas the response was weak or absent in the $CXCR3^+$ and $CCR4^+$ subsets, which are enriched in T_H1 and T_H2 cells, respectively (Fig. 2a). Similarly to what we observed in *in-vitro*-primed cultures, proliferating $CCR6^+CCR4^+$ memory T cells comprised both T_H17 and T_H1 cells, and *C. albicans*-specific T_H17 cells were mainly IL-17/IFN-γ double-producers, whereas *S. aureus*-specific T_H17 cells were IL-17 single- and IL-17/IFN-γ double-producers (Fig. 2b). Furthermore, upon restimulation with anti-CD3 and anti-CD28 or microbe-pulsed antigen-presenting cells, both *C. albicans*- and *S. aureus*-specific memory T_H17 clones downregulated IL-17 production, and *S. aureus*-specific clones, but not *C. albicans*-specific clones, upregulated IL-10 production with the same kinetics (Fig. 2c and Supplementary Fig. 3). Collectively, these findings demonstrate that the heterogeneous pattern of cytokine production and the regulated expression of IL-17 and IL-10 are characteristics shared by *in-vitro*- and *in-vivo*-primed microbe-specific T_H17 cells.

The analysis of memory T cells validated the *in vitro* priming system and prompted us to use this system to investigate the requirements for *C. albicans*- and *S. aureus*-induced T_H17 differentiation. In monocyte cultures, IL-6, TNF-α and IL-23 were elicited by both *C. albicans* and

[1]Institute for Research in Biomedicine, Via Vincenzo Vela 6, 6500 Bellinzona, Switzerland. [2]"G. Gaslini" Scientific Institute, Largo G. Gaslini 5, 16147, Genova, Italy. [3]Institute of Microbiology, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland. †Present address: Department of Dermatology and Berlin-Brandenburg School for Regenerative Therapies, Charité-Universitätsmedizin Berlin, Germany.

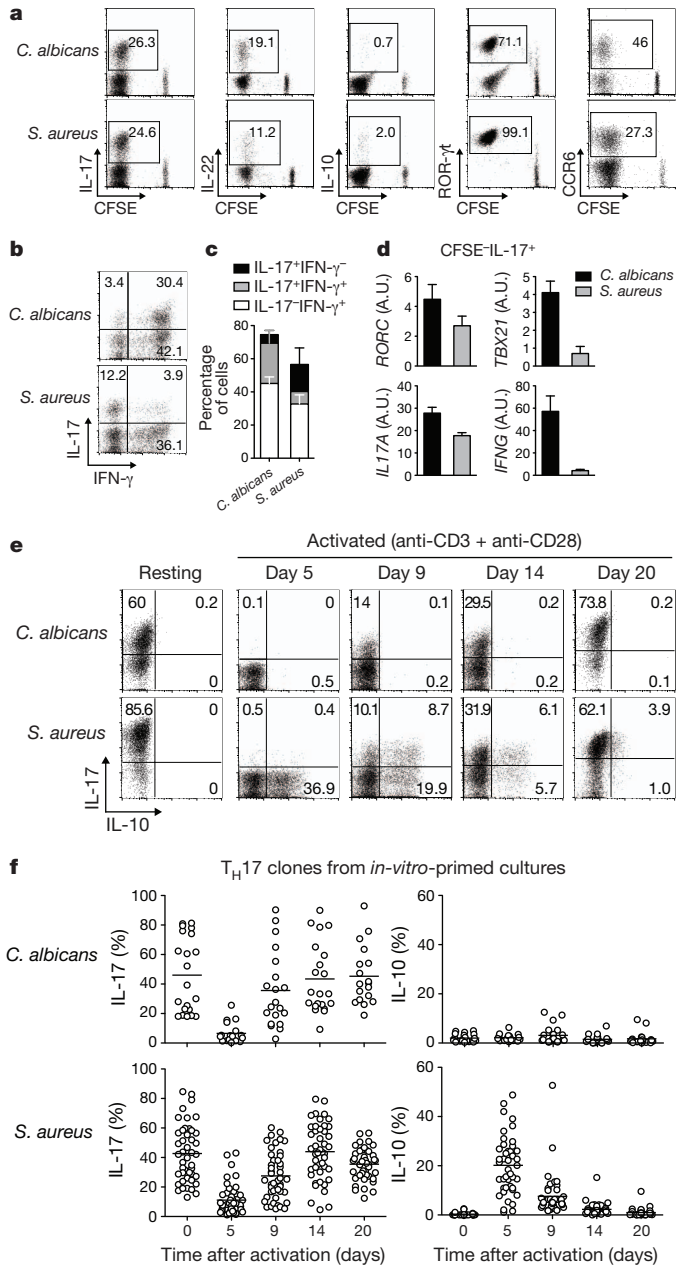

Figure 1 | *In vitro* priming of human naive T cells with *C. albicans* or *S. aureus* induces T_H17 cells that produce either IFN-γ or IL-10. **a**, Naive CFSE-labelled $CD4^+$ T cells were cultured with autologous monocytes pulsed with heat-inactivated *C. albicans* or *S. aureus*. On day 12, cells were stained with antibodies to ROR-γt and CCR6, or stimulated for 5 h with phorbol 12-myristate 13-acetate (PMA) and ionomycin (PMA+I), fixed, permeabilized, and stained with antibodies to IL-17, IL-22 and IL-10. Shown is a representative experiment and pooled data from several experiments are shown in Supplementary Fig. 1. **b**, **c**, IL-17 and IFN-γ production by $CFSE^-$ T cells primed by *C. albicans* or *S. aureus* as above. A representative staining is shown in **b** and pooled data are shown in **c** (mean and s.d., $n = 3$). **d**, *RORC*, *IL17A*, *TBX21* (which codes for T-bet) and *IFNG* mRNA expression assessed by quantitative RT–PCR in sorted IL-17-secreting cells. Mean and s.e.m. of triplicates are shown. Data are representative of two independent experiments. A.U., arbitrary units. **e**, **f**, *C. albicans*- or *S. aureus*-primed T cells were cloned by limiting dilution. T_H17 clones were selected and analysed in the resting state (day 0) and at different time points after restimulation with anti-CD3 and anti-CD28 for their capacity to produce IL-17 and IL-10 in response to PMA+I. A representative staining is shown in **e** and data from several clones (circles) and mean values (bars) are shown in **f**. Data are representative of more than five independent experiments. The amount of IL-17 and IL-10 secreted by *S. aureus*-specific T_H17 clones measured on day 5 by ELISA was 1,189 pg ml^{-1} (range 484–1,880) and 3,366 pg ml^{-1} (range 870–6,395), respectively. IL-17 production by *C. albicans*-specific T_H17 clones was 1,312 pg ml^{-1} (range 90–1,972). T_H1 clones isolated from the same *in-vitro*-primed cultures and restimulated under the same conditions did not upregulate IL-10 production, neither downregulated IFN-γ production.

S. aureus, whereas IL-1β was preferentially induced by *C. albicans* and IL-12p70 was detectable only in *C. albicans*-stimulated cultures (Supplementary Fig. 4). Neutralization of IL-6 or IL-23 partially inhibited T_H17 differentiation induced by both microbes, whereas neutralization of TGF-β modestly inhibited *C. albicans*-induced and enhanced *S. aureus*-induced T_H17 differentiation (Fig. 3a). In *S. aureus*-stimulated cultures neutralization of IL-1β had only a marginal effect but showed an additive effect when combined to neutralization of IL-6 and IL-23. In contrast, in *C. albicans*-stimulated cultures, neutralization of IL-1β strongly inhibited ROR-γt induction and differentiation of IL-17/IFN-γ double-producing T_H17 cells (Fig. 3a–c). Taken together, these findings indicate that IL-6, IL-23 and IL-1β contribute to T_H17 differentiation induced by both *C. albicans* and *S. aureus*, and reveal an absolute requirement for IL-1β in *C. albicans*- but not *S. aureus*-primed cultures.

To address the IL-1β-dependency of *C. albicans*-induced T_H17 differentiation, we examined the role of IL-12, which was selectively induced by *C. albicans*. Surprisingly, in *C. albicans*-stimulated cultures the combined neutralization of IL-1β and IL-12 restored priming of IL-17-producing cells, whereas neutralization of IL-12 alone enhanced priming of IL-17/IFN-γ double-producing cells (Fig. 3d). Conversely, addition of exogenous IL-12 to *S. aureus*-stimulated cultures inhibited in a dose-dependent fashion T_H17 differentiation, an effect that was significantly counteracted by IL-1β (Supplementary Fig. 5). Finally, priming with *S. aureus* in the presence of IL-1β induced T_H17 cells that produced IL-17 and IFN-γ and failed to upregulate IL-10 after restimulation (Fig. 3e, f). Taken together, these results indicate that IL-1β has multiple effects on T_H17 differentiation: it counteracts the T_H17-inhibitory activity of IL-12, induces IL-17/IFN-γ double-producing cells in an IL-12-independent manner, and inhibits IL-10 production capacity.

To establish whether IL-1β can induce IFN-γ and suppress IL-10 production in already differentiated T_H17 cells, we stimulated T_H17 clones or $CCR6^+CCR4^+$ memory T cells in the presence or absence of IL-1β and other innate cytokines. As shown in Supplementary Fig. 6a, IL-1β induced IFN-γ production in T_H17 clones to a level comparable to that induced by IL-12 (ref. 14), and suppressed IL-10 production. Furthermore, IL-1β significantly inhibited IL-10 production by $CCR6^+CCR4^+$ memory T cells, this inhibition being dominant over the enhancing effect of IL-12, IL-23 and IL-27, while it slightly enhanced IL-17 production (Fig. 3g and Supplementary Fig. 6b). In addition, IL-1β inhibited IL-10 production by $CXCR3^+$ and $CCR4^+$ memory T cell subsets (Fig. 3g). To assess the role of IL-1β in the regulation of IL-10 production *in vivo*, we isolated memory T cell subsets from patients with cryopyrin associated periodic syndrome (CAPS), an inflammatory disease caused by excessive production of IL-1β. IL-10 production was significantly lower in memory T cells and in T_H17 clones isolated during active disease compared to memory cells and clones isolated 7–10 days after *in vivo* administration of an IL-1RI antagonist (Fig. 3h and Supplementary Fig. 7). Taken together, these findings support the notion that IL-1β is a potent and general inhibitor of IL-10 production by memory T cells *in vitro* and *in vivo*.

A new finding emerging from this study is the transient downregulation of IL-17 production in recently activated T_H17 cells. To ask whether this is a general property of T_H17 cells and to investigate the underlying mechanisms we analysed a large number of random memory T_H17 clones (Supplementary Fig. 8). In virtually all clones, IL-17 production was downregulated on day 5 following restimulation and recovered at later time points, while IL-10 production was transiently upregulated in a fraction of the clones. In contrast, IL-22 and IFN-γ were produced in a stable fashion, irrespective of the activation state. Furthermore, the reciprocal IL-17/IL-10 regulation was observed

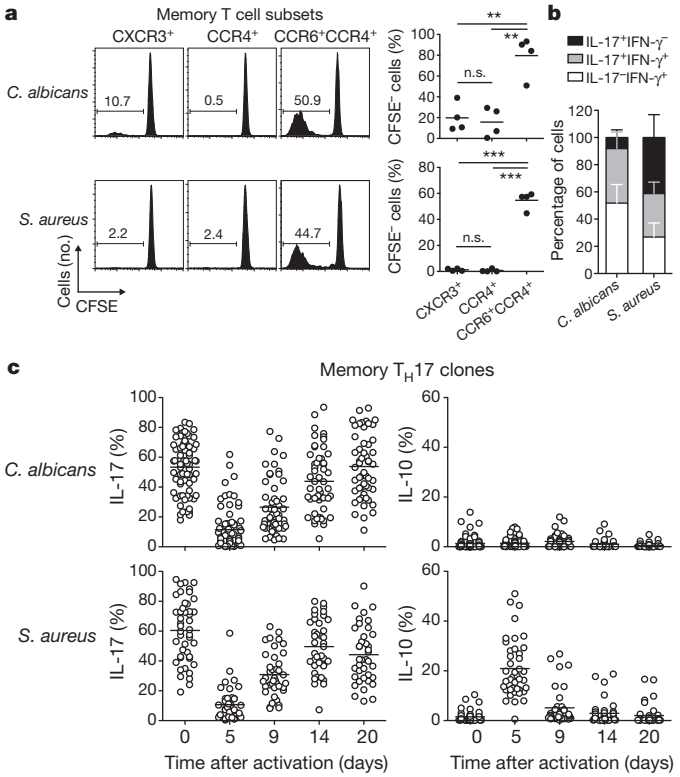

Figure 2 | Cytokine production by *C. albicans*- and *S. aureus*-specific memory T_H17 cells. **a**, $CXCR3^+CCR4^-CCR6^-$ ($CXCR3^+$), $CCR4^+CXCR3^-CCR6^-$ ($CCR4^+$), $CCR6^+CCR4^+CXCR3^-$ ($CCR6^+CCR4^+$) memory $CD4^+$ T cells were isolated from immune donors, labelled with CFSE and stimulated with autologous monocytes pulsed with *C. albicans* or *S. aureus*. Shown is the CFSE profile on day 5 in one representative donor and the percentage of $CFSE^-$ proliferating cells in four donors (**$P < 0.005$, ***$P < 0.0005$). **b**, Production of IL-17 and IFN-γ by proliferating $CCR6^+CCR4^+$ $CFSE^-$ cells measured by intracellular staining following PMA+I stimulation (mean and s.d. of three independent experiments). **c**, *S. aureus*- and *C. albicans*-specific memory T_H17 clones isolated from the $CCR6^+CCR4^+$ subset were analysed by intracellular cytokine staining before and at different time points after restimulation with anti-CD3 and anti-CD28. Data from several clones (circles) and mean values (bars) are shown. Data are representative of five independent experiments. The amount of IL-17 and IL-10 secreted by *S. aureus*-specific memory T_H17 clones measured on day 5 by ELISA was 1,263 pg ml^{-1} (range 349–2,241) and 6,134 pg ml^{-1} (range 1,358–14,160), respectively. IL-17 production by *C. albicans*-specific memory T_H17 clones was 1,850 pg ml^{-1} (range 94–6,189).

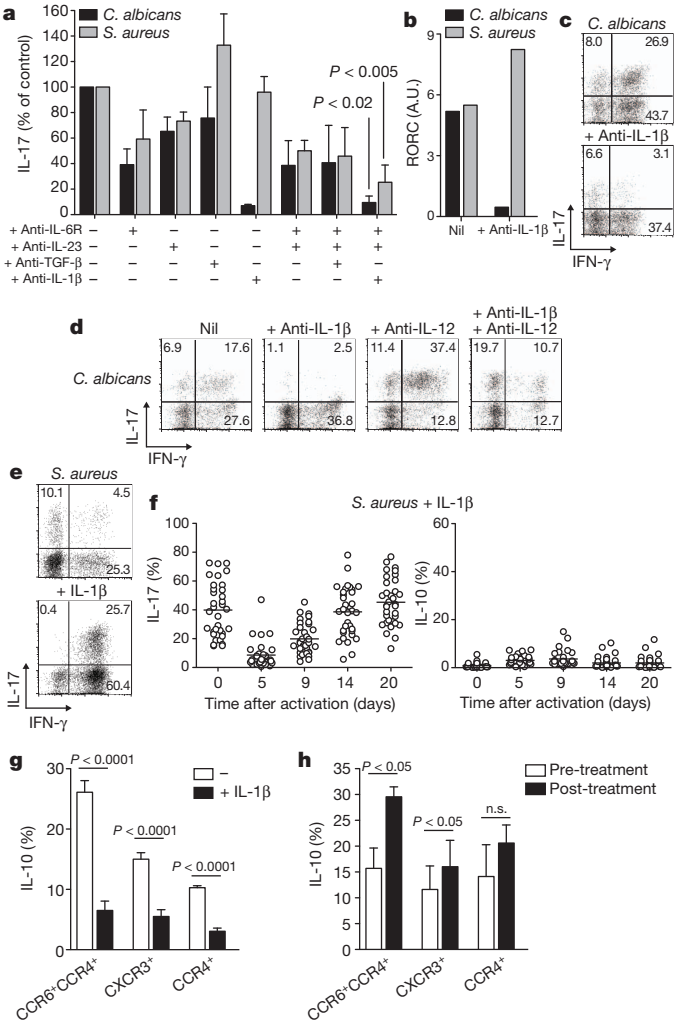

Figure 3 | Cytokine requirements for T_H17 differentiation induced by *C. albicans* and *S. aureus*. **a**, Naive $CD4^+$ T cells were primed by *C. albicans* or *S. aureus*-pulsed monocytes in the presence or absence of the indicated blocking antibodies. On day 12, the cells were stimulated with PMA+I and stained with antibodies to IL-17. Data are expressed as percentage of IL-17-producing cells relative to control cultures performed in the absence of neutralizing antibodies (mean and s.e.m., *n* ranging from 3 to 15). *P* values refer to control cultures. **b**, *RORC* mRNA expression in $CFSE^-$ T cells primed in the presence or absence of IL-1β-neutralizing antibodies. **c**, **d**, IL-17 and IFN-γ production by T cells primed with *C. albicans* in the absence or presence of neutralizing antibodies to IL-1β, IL-12 or both. **e**, IL-17 and IFN-γ production by T cells primed with *S. aureus* in the absence or presence of 10 ng ml^{-1} IL-1β. **f**, IL-17 and IL-10 production by resting and restimulated T_H17 clones isolated from cultures of T cells primed with *S. aureus* in the presence of 10 ng ml^{-1} IL-1β. Data in **b**–**f** are representative of at least three different experiments. **g**, Production of IL-10 by $CCR6^+CCR4^+$, $CXCR3^+$ and $CCR4^+$ memory $CD4^+$ T cells which were stimulated for 5 days with anti-CD3 and anti-CD28 in the presence or absence of 10 ng ml^{-1} IL-1β. Intracellular cytokine staining was performed on day 5 following PMA+I stimulation. Data are mean and s.e.m. of three independent experiments. **h**, Production of IL-10 by $CCR6^+CCR4^+$, $CXCR3^+$ and $CCR4^+$ memory $CD4^+$ T cells isolated from three CAPS patients at a time of active disease (pre-treatment) and 7 or 10 days after therapy with the IL-1RI antagonist anakinra (post-treatment), stimulated with anti-CD3 and anti-CD28 and tested on day 5 by intracellular cytokine staining following PMA+I stimulation. Data are mean and s.e.m.. Production of IL-17, IFN-γ and IL-4 by $CCR6^+CCR4^+$, $CXCR3^+$ and $CCR4^+$ memory T cells, respectively, was not significantly different in the pre- and post-treatment samples. n.s., not significant.

over repeated cycles of restimulation and its extent was dependent on the strength of T-cell receptor (TCR) stimulation. The analysis of transcription factors showed that on days 2 and 5 following restimulation, T_H17 clones downregulated expression of *RORC* mRNAs (which encode ROR-γt), concomitant with downregulation of *IL17A* mRNA (Fig. 4a). In addition, although both resting and day 5 restimulated T_H17 clones phosphorylated STAT3 in response to IL-6, only restimulated clones phosphorylated STAT5 in response to IL-2, consistent with the increased expression of CD25 (Fig. 4b–d). Overexpression of ROR-γt significantly restored IL-17 production in activated T_H17 clones, and restimulation in the presence of a STAT5 inhibitor, or an IL-2 neutralizing antibody, rescued *RORC* mRNA expression and IL-17 production in a proportion of clones (Fig. 4e–h and Supplementary Fig. 9). These findings indicate that decreased ROR-γt expression and increased pSTAT5 levels, that may compete with pSTAT3 for binding to the *IL17A* locus[15], contribute to the transient downregulation of IL-17 production in activated T_H17 cells.

The coherent picture that emerges from the analysis of *in-vitro*-primed and *ex vivo* memory cells indicates that *C. albicans* and *S. aureus* elicit different types of T_H17 cells that produce either IFN-γ or IL-10. These results extend in a relevant microbial system previous observations on the role of IL-6, IL-23, IL-1β and TGF-β in induction of human T_H17 cells[16–21] and reveal a role for IL-1β in counteracting

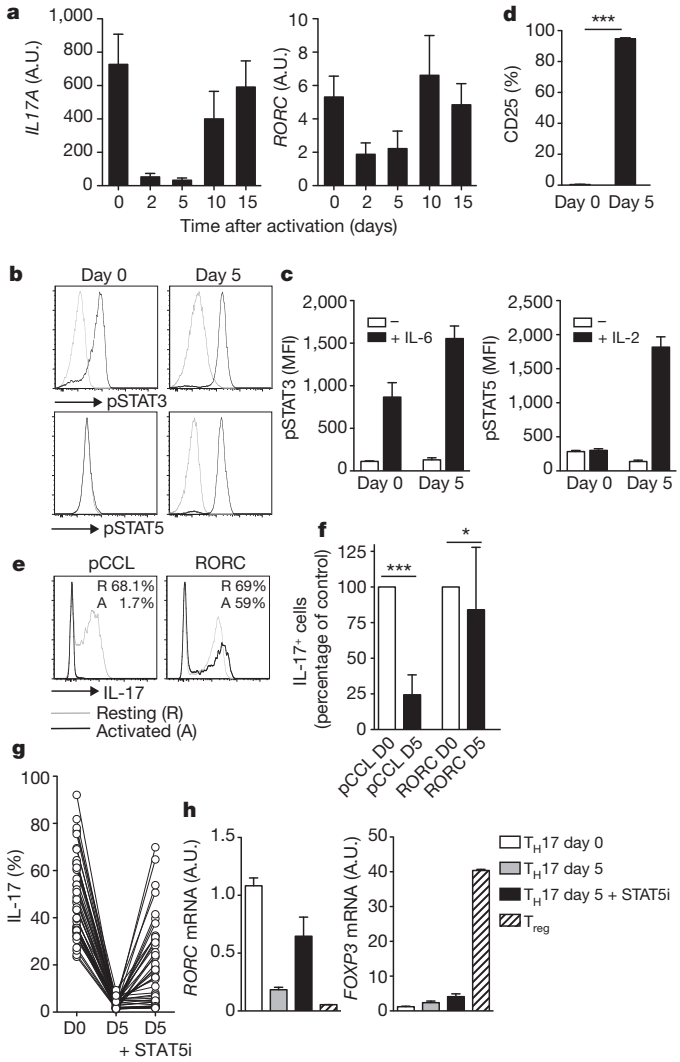

Figure 4 | Downregulation of ROR-γt and IL-2-mediated activation of STAT5 limit IL-17 production in activated T_H17 clones. **a**, T_H17 clones isolated from the $CCR6^+CCR4^+$ memory T cell subset were restimulated with anti-CD3 and anti-CD28. At the indicated time points, cells were stimulated with PMA+I for 3 h, and *IL17A* and *RORC* mRNAs were measured by quantitative RT–PCR. Data represent mean and s.e.m. of ten clones analysed. **b**, **c**, Resting (day 0) and day 5 restimulated T_H17 clones were exposed to IL-6 (50 ng ml^{-1}) or IL-2 (500 IU ml^{-1}) for 10 min and stained with antibodies to pSTAT3 and pSTAT5. A representative staining is shown in **b** (thin line, background staining) and mean and s.e.m. of four clones is shown in **c**. MFI, mean fluorescence intensity. **d**, Percentage of $CD25^+$ cells (mean and s.e.m.) in resting and day 5 restimulated T_H17 clones ($n = 8$, $^{***}P < 0.0005$). **e**, **f**, T_H17 clones were stably transfected with an empty lentiviral vector (pCCL) or with a vector containing the human *RORC* variant 2 (*RORC*). Percentage of $IL\text{-}17^+$ cells was measured in resting (day 0, thin line, empty bars) or in day 5 restimulated clones (thick line, black bars) 5 h after stimulation with PMA+I. Data shown are from two representative T-cell clones (**e**) and mean and s.e.m. of 12 pCCL-transfected and 28 *RORC*-transfected clones (**f**). $^*P < 0.05$, $^{***}P < 0.0005$. **g**, T_H17 clones were restimulated with anti-CD3 and anti-CD28 in the absence or presence of 100 μM STAT5 inhibitor N′-[(4-oxo-4H-chrome-3-yl)methylene]nicotinohydrazide. IL-17 production was measured in resting (day 0) and in day 5 clones restimulated in the absence or presence of the STAT5 inhibitor (STAT5i). Data are from 44 clones in one experiment and are representative of three independent experiments. **h**, T_H17 clones that showed sensitivity to STAT5 inhibition were analysed for *RORC* ($n = 8$) and *FOXP3* ($n = 14$) mRNA levels. Peripheral blood $CD4^+CD25^+$ regulatory T cells (T_{reg}) were included as control. Data are representative of three experiments. Note that *FOXP3* mRNA was not induced in T_H17 clones.

the T_H17-inhibitory effect of IL-12 and in promoting the differentiation of 'inflammatory' T_H17 cells that produce IL-17 and IFN-γ but not IL-10. The role of IL-1β in *C. albicans*-induced T_H17 differentiation is also supported by the analysis of IL-1β-deficient mice which showed a severe reduction in the T_H17 response to *C. albicans* and only a modest reduction in the T_H17 response to *S. aureus* (F.R., unpublished). Thus, our findings reveal a robust mechanism of microbe-induced T-cell differentiation that is dependent on the balance between polarizing cytokines rather than their absolute amounts (Supplementary Fig. 10). Our results also show that IL-1β can inhibit IL-10 production in memory T_H17 cells, a property that extends to T_H1 and T_H2 cells. The mechanism for this inhibition remains to be established although preliminary experiments suggest that NF-κB activation is not required (D.A., unpublished results). It is therefore possible that IL-1β may act not only at priming in lymphoid organs, but also at the effector phase in target tissues, where it may increase T cell inflammatory activity in synergy or antagonism with other factors that have been shown to modulate inflammation[12,22,23].

This study also reveals a new type of regulation whereby T_H17 cells continuously stimulated by antigen downregulate production of IL-17 while still producing IL-22 and, in some cases, upregulating IL-10, which together exert tissue protective and immunosuppressive effects. Inhibition of IL-17 production in activated T_H17 cells represents a new regulatory function of IL-2 besides its role in inhibiting T_H17 differentiation[24] and in the development, survival and function of regulatory T cells[25].

In conclusion, our study illustrates the feasibility of the *in vitro* and *ex vivo* combined approach to dissect the complexity of the human T-cell response to microbes and reveals novel mechanisms for differentiation of stable subsets of T_H17 cells endowed with different inflammatory capacity as well as factors that modulate their effector function.

METHODS SUMMARY

Cell sorting. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque Plus (GE Healthcare). T cells and monocytes were isolated with CD4 and CD14 microbeads (Miltenyi Biotech), respectively. Naive T cells were sorted as $CD45RA^+CD45RO^-CCR7^+CD25^-CD8^-$. Memory $CD45RA^-CD25^-CD8^-$ T cell subsets were sorted according to the differential expression of CXCR3, CCR4 and CCR6. Viable $IL\text{-}17^+$ *in-vitro*-differentiated T cells were FACS-sorted using the cytokine secretion assay (Miltenyi Biotech) after 3-h stimulation with PMA and ionomycin.

T cell assays and cloning. CFSE-labelled naive $CD4^+$ T cells were co-cultured for 12 days with autologous irradiated monocytes (2:1 ratio) that were pre-incubated for 3 h with heat-inactivated *C. albicans* or *S. aureus*. Some experiments were performed in the presence of neutralizing antibodies or cytokines. Antigen-specific T-cell clones from *in vitro*-primed T cells or from *in vitro*-restimulated memory T cells were generated by sorting CFSE-negative cells (day 12 for T cells primed *in vitro*, day 5 for memory T cells), followed by expansion for 7 days in IL-2-containing media (50 U ml^{-1}) and cloning by limiting dilution. Random T_H17 clones were generated by direct cloning of $CCR6^+CCR4^+$ memory $CD4^+$ T cells. Resting (>day 20 after stimulation) T-cell clones were activated for 48 h with anti-CD3 (1 μg ml^{-1}, TR66) and anti-CD28 (1 μg ml^{-1} CD28.2; BD Biosciences) or *C. albicans*- or *S. aureus*-pulsed autologous monocytes and analysed at different time points.

Cytokine and transcription factor analysis. Intracellular staining for cytokines and transcription factors was performed as described in detail in Methods. Cytokine secretion by monocytes was analysed using the cytometric bead array (BD Biosciences). Cytokine and transcription factor gene expression was analysed by quantitative reverse transcription PCR (RT–PCR; all probes from Applied Biosystems).

Statistics. Student's two-tailed paired *t*-test was used for statistical comparisons; *P* values of 0.05 or less were considered as significant.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions C.E.Z. designed and performed experiments, analysed the data and wrote the manuscript, D.J. performed the sorting and analysed data, F.R. performed and analysed experiments in the mouse system, F.M., D.A., and S.M. performed experiments to address the mechanism of IL-17 downregulation and analysed the data, M.G. provided the samples from CAPS patients and analysed the data, A.L. wrote the manuscript, F.S. provided overall supervision, analysed the data and wrote the manuscript.

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METHODS

Blood samples and cell sorting. Blood from healthy donors was obtained from the Swiss Blood Donation Center of Basel and Lugano, and used in compliance with the Federal Office of Public Health (authorization no. A000197/2 to F.S). Peripheral blood was collected from three CAPS patients carrying the T50M, D303N and M406I mutation of the *NLRP3* gene[26] at a time of active disease and 7–10 days after treatment with the IL-1RI antagonist anakinra, after receiving the patient's informed consent and approval by the "G. Gaslini" Ethical board. $CD14^+$ monocytes and $CD4^+$ T cells were isolated from PBMC by positive selection using magnetic microbeads (Miltenyi Biotec). T helper cell subsets were sorted to over 97% purity as follows: $CXCR3^+CCR4^-CCR6^-CD45RA^-CD25^-CD8^-$ (enriched in T_H1 cells); $CCR4^+CXCR3^-CCR6^-CD45RA^-CD25^-CD8^-$ (enriched in T_H2 cells); $CCR6^+CCR4^+CXCR3^-CD45RA^-CD25^-CD8^-$ (enriched in T_H17 cells). The following antibodies were used for FACS-sorting and analysis: anti-CCR6–phycoerythrin (PE) (11A9), anti-CCR4–PE–cyanin 7 (Cy7) (1G1), anti-CXCR3–allophycocyanin (APC) (1C6), anti-CD45RA–PE–Cy5 (HI100; all from BD Biosciences), anti-CD8–PE–Cy5 (B9.11), anti-CD25–FITC (fluorescein isothiocyanate) (B1.49.9; both from Immunotech), and anti-ROR-γt (600380; R&D Systems, AFKJS-9; eBioscience). Naive T cells were isolated as $CD45RA^+CD45RO^-CCR7^+CD25^-CD8^-$ to a purity of over 99% after staining with anti-CD45RA, anti-CD25 and anti-CD8 as well as anti-CD45RO–FITC (UCHL1; Immunotech) and anti-CCR7 (150503; R&D Systems), followed by staining with biotinylated anti-IgG2a (1080-08; Southern Biotech) and streptavidin–Pacific blue (Molecular Probes; Invitrogen). Cells were sorted with a FACSAria (BD Biosciences). Viable $IL\text{-}17^+$ cells were FACS-sorted using the cytokine secretion assay (Miltenyi Biotec).

T-cell culture. Cells were cultured in RPMI-1640 medium supplemented with 2 mM glutamine, 1% (v/v) non-essential amino acids, 1% (v/v) sodium pyruvate, penicillin (50 U ml^{-1}), kanamycin (50 μg ml^{-1}), streptomycin (50 μg ml^{-1}; all from Invitrogen) and 5% (v/v) human serum (Swiss Blood Center). Monocytes were pre-incubated for 3 h with *C. albicans* (ratio of 1:3) or *S. aureus* (5 μg ml^{-1}) and irradiated (45 Gy) before T-cell co-culture. Microbes were killed by heating at 65 °C for 1 h according to standard methods, followed by three freeze-thaw cycles for *S. aureus*. Protein concentration was determined by the bicinchoninic acid assay (Bio-Rad) according the manufacturer's instructions. The concentration of inactivated microorganisms used in co-culture experiments was determined after titration in proliferation assays using ^{3}H-thymidine incorporation, as described previously[27]. The concentration leading to maximum proliferative responses and viability was chosen. T cells were labelled with CFSE according to standard protocols. For T-cell priming experiments, naive CFSE-labelled $CD4^+$ T cells (5×10^4) were co-cultured with autologous monocytes at a ratio of 2:1 in the absence of exogenous IL-2 for 12 days before analysis. In these primary cultures microbes did not induce apoptosis of monocytes (in one representative experiment, the percentage of annexin-V^+ cells in cultures of monocytes alone, monocytes and *C. albicans*, monocytes and *S. aureus* was 16.9, 18.3 and 16.5, respectively). For recall responses, CFSE-labelled purified memory T helper cell subsets were co-cultured with irradiated autologous monocytes at a ratio of 2:1 for 5 days before analysis. Some experiments were performed using glutaraldehyde-fixed *C. albicans*- or *S. aureus*-pulsed monocytes with similar results. Antigen-specific T-cell clones derived from *in-vitro*-primed T cells as well as from memory T cells were generated as previously described[28]. Briefly, CFSE-negative cell populations (day 12 for T cells primed *in vitro*, day 5 for memory T cells) were sorted, expanded for 7 days in IL-2-containing media (50 U ml^{-1}) and cloned by limiting dilution using phytohaemagglutinin (PHA, 1 μg ml^{-1}; Remel), irradiated (45 Gy) allogeneic feeder cells and IL-2. Antigen specificity of T-cell clones was confirmed by their capacity to proliferate in response to irradiated autologous monocytes pulsed with the respective microbe, as described previously[27]. T-cell clones were stimulated in the resting state (25–30 days after the first stimulation or as indicated) for 48 h with plate-bound anti-CD3 (1–5 μg ml^{-1}, clone TR66) and anti-CD28 (1 μg ml^{-1} CD28.2; BD Biosciences) or with autologous antigen-presenting cells (irradiated monocytes or Epstein–Barr virus-immortalized B cells) and microbes. In some experiments T-cell cultures were performed in the presence of neutralizing antibodies or exogenous cytokines. The following neutralizing antibodies were used (all at 10 μg ml^{-1}): anti-IL-1β (8516), anti-IL1R1 (AF269), anti-IL-6R (17506), anti-IL-23 p19 (AF1716), anti-TGF-β (1D11) (all from R&D Systems), anti-IL-12p70 (20C2, BD Biosciences), or anti-human leukocyte antigen (HLA)-DQ (SPVL-3), anti-HLA-DP (B7/21), anti-HLA-DR (L243; all from ATCC). In preliminary experiments the IL-12p70 antibody 20C2 was found to inhibit pSTAT4 phosphorylation induced by IL-12 while not affecting pSTAT3 phosphorylation induced by IL-23 (F.M., unpublished).

Cytokine analysis. For intracellular cytokine staining, cells were restimulated for 5 h with PMA and ionomycin (PMA+I) in the presence of brefeldin A (all from Sigma-Aldrich) for the final 2.5 h of culture. Cells were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's instructions. Cells were stained with anti-IL-10 (JES3-19F1; BD Biosciences), anti-IL-17 (eBIO64-DEC17; eBioscience), anti-IFN-γ (B27; BD Biosciences), and anti-IL-22 (142928; R&D Systems), conjugated with different fluorochromes, and were analysed on a FACSCalibur (BD Bioscience). Flow cytometry data were analysed with FlowJo (Tree Star). IL-1β, IL-6 and TNF-α and IL-12 secretion by monocytes was measured by cytometric bead array (BD Biosciences) or Luminex (Invitrogen) according to the manufacturer's instructions.

STAT analysis. T_H17 clones were restimulated with anti-CD3 (5 μg ml^{-1}) and anti-CD28 (1 μg ml^{-1}). After a total incubation time of 96 h, cells were extensively washed and incubated for a further 24 h. At the end of the incubation time, cells were again extensively washed and stimulated for 10 min at 37 °C with recombinant IL-6 (50 ng ml^{-1}, R&D Systems) or recombinant IL-2 (500 U ml^{-1}, produced in our laboratory). Cells were then fixed at 37 °C for 30 min using 3.5% formaldehyde and permeabilized using 90% methanol and stained for pSTAT3 and pSTAT5 using fluorophore-conjugated antibodies (clones D3A7 an C71E5, respectively, both from Cell Signalling Technology) and analysed by FACS.

Gene expression analysis. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Random hexamer primers and a Moloney murine leukemia virus (MMLV) reverse transcriptase kit (Stratagene) were used for cDNA synthesis. Transcripts were quantified by RT–PCR on an ABI PRISM 7700 Sequence Detector with predesigned TaqMan Gene Expression Assays (*IL17A* Hs99999082_m1, *RORC* Hs01076112_m1, *TBX21* Hs00203436_m1, *FOXP3* Hs01085834_m1) and reagents according to the manufacturer's instructions (Applied Biosystems). For each sample, expression of target genes was normalized to 18S ribosomal RNA (Applied Biosystems) and expressed as arbitrary units (A.U.).

Plasmids and lentiviral transduction. The vectors pCLL (empty) and the pCCL.RORC2 (containing the cDNA encoding for the human RORC variant 2)[29] were provided by M. Levings. Both vectors also expressed the ΔNGFR reporter gene as a marker for transduction. Lentiviral particles were produced by transiently transfecting 293FT cells with the pCCL or pCCL.RORC2 transfer vectors together with the packaging vectors psPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259), as previously described[30]. Briefly, 293FT cells were transfected with a cocktail of transfer vector, psPAX and pMDG2.G at a ratio 4:3:1 in Opti-MEM, using linear polyethylenimine as transfecting agent. Viral particles were harvested at 36 and 48 h post-transfection, concentrated by centrifugation on a sucrose gradient, and titres were determined by limiting dilution on 293FT cells. Concentrated lentivirus was added to human memory T_H17 cell lines at a multiplicity of infection of ~60 in the presence of 500 U ml^{-1} recombinant IL-2. Three days later, the efficiency of transduction was determined by surface staining for NGFR using a biotinylated anti-CD127 antibody (BD Biosciences), and $NGFR^{hi}$ cells were FACS-sorted and cloned. Two weeks later, 30 pCCL control clones and 96 pCCL.RORC2 clones were assessed for NGFR and IL-17 expression. The pCLL-transduced clones were 90% $NGFR^+$, of which 40% expressed IL-17, whereas 50% of the pCCL-RORC2 clones were $NGFR^+$, of which 82% expressed IL-17. Three weeks after transduction, IL-17-producing clones (12 for pCCL and 28 for pCCL.RORC2) were either left resting or were restimulated for 48 h on plate-bound anti-CD3 (5 μg ml^{-1} TR66) and anti-CD28 (1 μg ml^{-1}), and intracellular cytokine staining for IL-17 expression was performed 5 days later.

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