



OVER 200 ACTIVE HUMAN, MOUSE and RAT PROTEINS in 4 CONVENIENT SIZES.

Phone: **877.613.6020** • Fax: **877.617.9530**
www.shenandoah-bt.com



Cutting Edge: IFN- γ Regulates the Induction and Expansion of IL-17-Producing CD4 T Cells during Mycobacterial Infection

This information is current as of November 28, 2011

Andrea Cruz, Shabaana A. Khader, Egidio Torrado, Alexandra Fraga, John E. Pearl, Jorge Pedrosa, Andrea M. Cooper and Ant3nio G. Castro

J Immunol 2006;177;1416-1420

References This article **cites 38 articles**, 18 of which can be accessed free at:
<http://www.jimmunol.org/content/177/3/1416.full.html#ref-list-1>

Article cited in:
<http://www.jimmunol.org/content/177/3/1416.full.html#related-urls>

Subscriptions Information about subscribing to *The Journal of Immunology* is online at
<http://www.jimmunol.org/subscriptions>

Permissions Submit copyright permission requests at
<http://www.aai.org/ji/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at
<http://www.jimmunol.org/etoc/subscriptions.shtml/>



Cutting Edge: IFN- γ Regulates the Induction and Expansion of IL-17-Producing CD4 T Cells during Mycobacterial Infection¹

Andrea Cruz,* Shabaana A. Khader,[†] Egidio Torrado,* Alexandra Fraga,* John E. Pearl,[†] Jorge Pedrosa,* Andrea M. Cooper,^{2†} and António G. Castro*

*T cell responses are important to the control of infection but are deleterious if not regulated. IFN- γ -deficient mice infected with mycobacteria exhibit enhanced accumulation of activated effector T cells and neutrophils within granulomatous lesions. These cells do not control bacterial growth and compromise the integrity of the infected tissue. We show that IFN- γ -deficient mice have increased numbers of IL-17-producing T cells following infection with *Mycobacterium bovis* bacille Calmette Guérin. Furthermore, exogenous IFN- γ increases IL-12 and decreases IL-23 production by bacille Calmette Guérin-infected bone marrow-derived dendritic cells and reduces the frequency of IL-17-producing T cells induced by these bone marrow-derived dendritic cells. These data support the hypothesis that, during mycobacterial infection, both IFN- γ - and IL-17-producing T cells are induced, but that IFN- γ serves to limit the IL-17-producing T cell population. This counterregulation pathway may be an important factor in limiting mycobacterially associated immune-mediated pathology. The Journal of Immunology, 2006, 177: 1416–1420.*

The immune system must mount a rapid response to invading pathogens while controlling this response. Inappropriate stimulation and/or failure to limit this response can result in tissue damage (1–4). Factors that limit inappropriate responses include negative selection of autoreactive T cells and innate response-mediated initiation of acquired immunity and regulatory T cells (2, 5–9). An additional control mechanism is negative feedback of inflammatory processes by inflammatory cytokines (10, 11). In this regard, during mycobacterial infection of IFN- γ -deficient mice, activated effector T cells and neutrophils are unregulated and result in a destructive granulomatous response (12–15).

A pathway of T cell activation, initiated by TGF β and IL-6 (16–18) and resulting in responsiveness to IL-23 (18) results in Ag-specific T cells that produce IL-17 (Th17). These cells are present during autoimmune diseases such as experimental autoimmune encephalitis and collagen-induced arthritis (19–21). IL-17 is an active recruiter of neutrophils to inflammatory sites (22–24) and IFN- γ regulates the induction of Th17 cells (25, 26). These facts may explain the damaging inflammatory response seen during mycobacterial infection of IFN- γ -deficient mice (15) in that IFN- γ and IL-17 may counterregulate each other during chronic mycobacterial infection.

IFN- γ - and IL-17-producing CD4 T cells are expanded by *Mycobacterium tuberculosis* (Mtb)³-infected dendritic cells (DC) and are induced in vivo during infection (27). Although both cell types are generated during primary progressing Mtb infection (27) counterregulation may be more readily observable during low-virulence mycobacterial infection, this type of infection also may better model the type of mycobacterial exposure that has been correlated with arthritis (28, 29).

To test whether counterregulation between IFN- γ and IL-17 occurs during low-virulence mycobacterial infection, we infected mice with bacille Calmette Guérin (BCG) and examined the kinetics of IFN- γ - and IL-17-producing cellular responses. We also tested the ability of IFN- γ to modulate the size of the IL-17-producing population and the ability of IFN- γ and IL-17 to alter the production of IL-12p70 and IL-23 by BCG-infected DC. Our data demonstrate that low-virulence mycobacterial infection induces a negative feedback loop whereby IFN- γ -producing cells limit IL-17-producing cells, and that this feedback loop is perturbed by the absence of IFN- γ .

Materials and Methods

Mice and experimental infection

C57BL/6 (B6) and IFN- γ -deficient (*B6.129S7-Ifng^{tm1Tz}*) were infected i.v. with 1×10^6 or 5×10^6 BCG Pasteur. CD4⁺ T cells were obtained from OT-II $\alpha\beta$ TCR transgenic (TCRTg) male mice (OT-II)(*C57BL/6-TgN* (*TcrOT-II*)) of ≤ 8 wk of age. Breeding stocks of IL-23-deficient (*B6.123a^{-/-}*)

*Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal; and [†]Trudeau Institute, Saranac Lake, NY 12983

Received for publication December 20, 2005. Accepted for publication June 6, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ A.C. was supported by the Fundação para a Ciência e Tecnologia Grant SFRH/BD/9624/2002 through the Graduate Program in Applied and Basic Biology Programme, University of Porto, Portugal. S.A.K. was supported by a New York Community Trust-Heiser

Fund Fellowship. Other funding came from the Health Service of Fundação Calouste Gulbenkian, Fundação para a Ciência e a Tecnologia (POCT1/ECP/49087/01), National Institutes of Health (AI46530), and the Trudeau Institute.

² Address correspondence and reprint requests to Dr. Andrea M. Cooper, Trudeau Institute, 154 Algonquin Avenue, Saranac Lake, NY 12983. E-mail address: acooper@trudeauinstitute.org

³ Abbreviations used in this paper: Mtb, *Mycobacterium tuberculosis*; DC, dendritic cell; BMDC, bone marrow-derived DC; BCG, bacille Calmette Guérin; TCRTg, TCR transgenic; MOI, multiplicity of infection.

mice were obtained from Dr. N. Ghilardi (Genentech) (27). All mice were bred at the Trudeau Institute and were used under its Institutional Animal Care and Use Committee guidelines.

Culture of murine bone marrow-derived DC (BMDC)

BMDC were derived from B6- and IL-23-deficient mice as described (27, 30). Briefly, cells were cultured in medium containing murine granulocyte monocyte CSF. On day 10 of culture, nonadherent cells were placed in 24-well plates and infected with BCG at a multiplicity of infection (MOI) of 2. Some cultures received 500 U/ml mouse IFN- γ (eBioscience) or 100 ng/ml recombinant mouse IL-17 (R&D Systems).

ELISA

Supernatants from BMDC culture were screened for IL12p70 and IL-23 using the mouse IL-12p70 Quantikine ELISA kit (R&D Systems) and the mouse IL-23 Ready Set-Go ELISA kit (eBioscience).

In vitro CD4⁺ T cell activation

Naive CD4⁺ T cells were generated from OT-II mice as described (27) and cultured (1×10^6 cells/ml) with BMDC (1×10^6 cells/ml) for 72 or 120 h at 37°C in 5% CO₂, in 10 ng/ml IL-2 and 5 μ M Ova₃₂₃₋₃₃₉ peptide. Some BMDC were infected with BCG at an MOI of 2. IFN- γ was used at 100 U/ml. Stimulated T cells were washed and counted, and the frequency of IL-17-producing CD4⁺ T cells determined by ELISPOT.

ELISPOT assay

ELISPOT was performed as described previously (27). Briefly, a total of 1×10^5 cells was added to Ab-coated wells, 2-fold dilutions were made, and irradiated splenocytes from B6 mice were added at 1×10^6 cells per well. A peptide representing an I-A^b-restricted epitope of Ag 85A (31) was used to stimulate cells from infected mice, whereas Ova₃₂₃₋₃₃₉ stimulated OT-II T cells (32); all wells contained 10 ng/ml IL-2. After 24 h, plates were washed and processed as described (27). Cells from mice infected with BCG, but cultured in the absence of Ag, did not produce spots.

Statistical analysis

The results are given as means \pm SE of the mean. Statistical significance was calculated by using Student's *t* test. Values of *p* < 0.05 were considered significant.

Results and Discussion

BCG infection induces both IFN- γ - and IL-17-producing cells

IFN- γ - and IL-17-producing cells are induced during progressive Mtb infection (27). However, the kinetics of these popula-

tions during a self-limiting mycobacterial infection are not known. We, therefore, infected mice with BCG, which is cleared by the host (33), and examined the kinetics of Ag-specific CD4⁺ T cell responses. Splenic lymphocytes from infected mice were stimulated in vitro with an I-A^b-restricted peptide of the dominant mycobacterial Ag, Ag85 (31), and their cytokine expression determined by ELISPOT. Using a low dose of BCG, we show that equal induction of IFN- γ - and IL-17-producing CD4 T cells occurs up to day 8 but that, by day 15, there is a decreased number of IL-17-producing cells, compared with IFN- γ -producing cells (Fig. 1*a*). In a second experiment with a higher dose of BCG, the same pattern is seen and extends to day 21 (Fig. 1*b*). The increased numbers of cells also is reflected in an increased frequency of these cells within the splenocytes culture (data not shown). These data suggest that, although mycobacterial infection can induce both IFN- γ - and IL-17-producing cells, the IFN- γ response may down-regulate the IL-17 response.

IFN- γ inhibits the development of IL-17-producing cells in response to mycobacterial infection

IFN- γ modulates the IL-17 response in vitro (25, 26) and in vivo (18); to test, therefore, whether IFN- γ production was limiting the IL-17 T cell response to BCG, we infected B6- and IFN- γ -deficient mice and compared the expansion of the IL-17-producing CD4 T cell population response over time. We

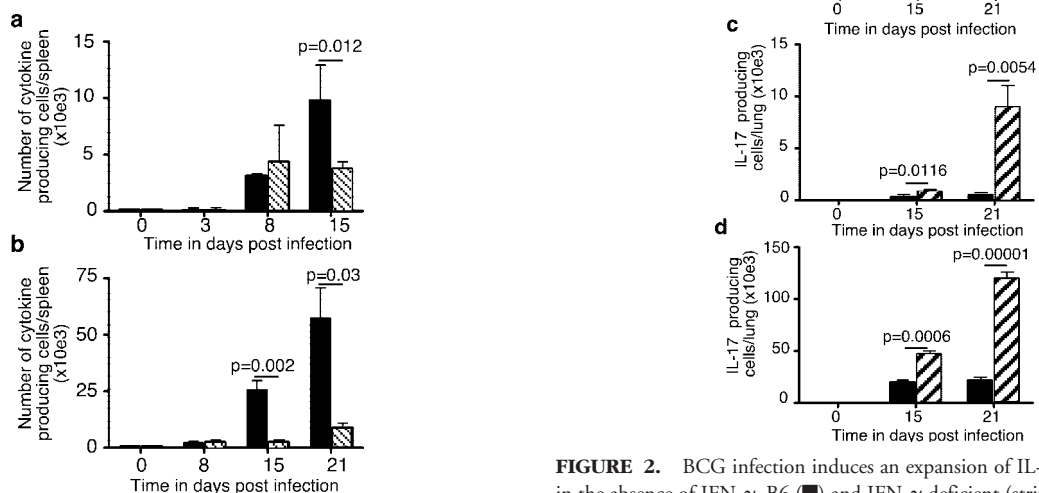


FIGURE 1. BCG infection induces the differentiation of both IFN- γ and IL-17-producing cells. B6 mice were infected i.v. with 1×10^6 (*a*) or 5×10^6 (*b*) CFU BCG. Splenocytes were stimulated with Ag85 peptide and IFN- γ (■) and IL-17 (striped bar) production assessed by ELISPOT. Data points represent the mean of *n* = 4. Significance was determined using Student's *t* test.

FIGURE 2. BCG infection induces an expansion of IL-17-producing cells in the absence of IFN- γ . B6 (■) and IFN- γ -deficient (striped bar) mice were infected i.v. with 4×10^6 CFU BCG and frequency of IL-17-producing cells specific for the Ag85 peptide (*a* and *c*) or BCG (*b* and *d*) in the spleen (*a* and *b*) or lung (*c* and *d*) assessed by ELISPOT. Data points represent the mean of *n* = 4, significance determined by Student's *t* test. Results are from one representative experiment of two independent experiments.

Table I. The frequency of IL-17-producing cells in BCG-infected mice is increased in the absence of IFN- γ

Ag ^a	Mouse Strain	Day Post Infection ^b	Frequency of IL-17-Producing Cells ^c	
			Spleen	Lung
Ag85	B6	15	2.7e-5 \pm 1.5e-6	3.2e-5 \pm 2.7e-6
		21	4.8e-5 \pm 1.1e-5	4.3e-5 \pm 1.2e-5
	IFN- γ Deficient	15	1.1e-4 \pm 3.5e-5***	5.5e-5 \pm 8.5e-6*
		21	2.6e-4 \pm 1.5e-4***	4.6e-4 \pm 1.1e-4***
BCG	B6	15	1.5e-4 \pm 9.0e-5	2.0e-4 \pm 7.6e-5
		21	2.6e-4 \pm 1.0e-4	1.7e-4 \pm 2.7e-5
	IFN- γ Deficient	15	5.3e-4 \pm 1.3e-5***	3.1e-4 \pm 1.3e-4**
		21	6.2e-4 \pm 1.4e-4***	6.4e-4 \pm 1.4e-4***

^a Single-cell suspensions from infected mice were cultured with either Ag85 peptide or BCG.

^b B6 or IFN- γ -deficient mice were infected i.v. with BCG and harvested on day 15 or 21.

^c The frequency of cytokine-producing cells was determined by ELISPOT.

*, $p = 0.078$, **, $p < 0.01$, ***, $p < 0.001$.

show that, although the Ag85 peptide-specific IL-17-producing cellular response in B6 mice is limited in both the spleen (Fig. 2a) and lung (Fig. 2c), this response expands to a significantly higher degree in the IFN- γ -deficient mice (Fig. 2, a and c). This expansion occurs despite the fact that the bacterial burden differs only slightly at day 15 (B6 $5.5 \pm 0.37 \log_{10}$; IFN- γ -deficient $6.17 \pm 0.12 \log_{10}$). This differential response in the IFN- γ -deficient mice also was seen when live BCG was used to restimulate cells (Fig. 2, b and d). The increased numbers of IL-17-producing cells per organ in the IFN- γ -deficient mice also were reflected in increased frequencies for these cells (Table I). These data support the hypothesis that IFN- γ regulates the extent of the IL-17 response during BCG infection.

IFN- γ and IL-17 counterregulate the production of IL-23 and IL-12p70 by mycobacteria-infected DC

Although Th1 cells require IL-12p70 for optimal induction in response to mycobacteria-infected BMDC, IL-17-producing cells require IL-23 (27), thus the impact of IFN- γ on the development of IL-17-producing cells may lie in its ability to modulate the production of these cytokines by APC. To test this, we measured whether IFN- γ or IL-17 could modulate the

production of IL-12p70 or IL-23. Although little IL-12p70 was detected from BCG-infected BMDC, this was increased in the presence of IFN- γ but not IL-17 (Fig. 3a). In contrast, IL-23 was detected in the BCG-infected BMDC supernatants, and this was significantly reduced in the presence of IFN- γ and increased by IL-17 (Fig. 3b). These data show that IFN- γ alters the cytokine-producing profile of BCG-infected BMDC to one that favors IFN- γ -producing cells over IL-17-producing cells, i.e., increased IL-12p70 and reduced IL-23.

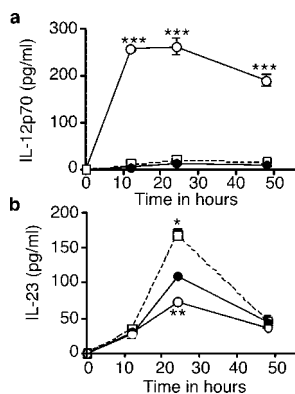


FIGURE 3. IL-17 up-regulates IL-23, but not IL-12p70. BMDC were infected with BCG at an MOI of 2 and either left untreated (●) or treated with IFN- γ (○) or IL-17 (dashed line, □). BMDC culture supernatants were harvested and analyzed by ELISA for IL-12p70 (a) and IL-23 (b). Data points represent the mean of $n = 3$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, relative to BCG alone) as determined by Student's t test. Results are from one representative experiment of three independent experiments.

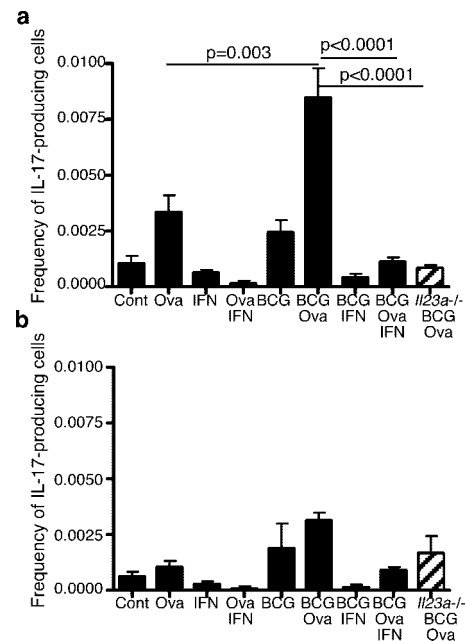


FIGURE 4. IFN- γ inhibits the differentiation of CD4⁺ T cells into IL-17-producing cells. a, Purified OT-II TCR transgenic CD4⁺ T cells were cultured for 5 days with BMDC from either B6 mice (■) or B6.IL23a^{-/-} mice (striped bars). The BMDC were either infected with BCG at an MOI of 2 or left uninfected. Some cultures contained the Ova₃₂₃₋₃₃₉-peptide (Ova) and/or IFN- γ . a, After 5 days, the cells were washed and the ability of cells to produce IL-17 in response to peptide determined by ELISPOT. b, The ability of cells from the 5-day culture to produce IL-17 spontaneously in the ELISPOT assay (i.e., without exogenous peptide) also was determined. Data points represent the mean of $n = 3$, significance was determined by Student's t test. Results are from one representative experiment of two independent experiments.

Addition of IFN- γ to BCG-infected BMDC reduces the frequency of IL-17-producing T cells

To test whether exogenous IFN- γ could impact the generation of IL-17-producing cells, we added IFN- γ to cultures of CD4⁺ TCRTg T cells being stimulated by BCG-infected BMDC. TCRTg T cells were used to obtain sufficient numbers of T cells of known Ag specificity. T cells were activated by BCG-infected BMDC pulsed with the cognate Ag, an OVA peptide consisting of residues 323–339 (OVA_{323–339}). The ability of these activated T cells to produce IL-17 was measured by ELISPOT. We show in this study that the presentation of Ag by BCG-infected BMDC dramatically increases the frequency of IL-17-producing cells, compared with that seen when uninfected BMDC are used (Fig. 4*a*). Thus, BCG infection of APC results in an increased polarization of cells to an IL-17-producing phenotype. In contrast, when IFN- γ was added to the BCG-infected BMDC cultures, there was a significant decrease in the frequency of IL-17-producing cells induced (Fig. 4*a*). This occurred despite the fact that there was not a reduction in total number of cells (OVA + BCG $1.12 \times 10^5 \pm 6.7 \times 10^3$ cells per well, compared with OVA + BCG + IFN $1.29 \times 10^5 \pm 7.6 \times 10^3$ cells per well; $n = 3$).

Recent publications suggest that IL-23 is not the primary factor in the initial polarization of IL-17-producing cells. Rather, TGF β initiates the IL-17-producing phenotype (16–18), and IL-6 modifies the action of TGF β such that IL-17-producing, rather than regulatory, T cells are induced (17). We, therefore, wanted to determine whether IL-23 played any role in mediating the generation of IL-17-producing cells when mycobacteria are used to activate the presenting cell. To do this, we used mice lacking the IL-23 subunit p19 (B6.*Il23a*^{-/-}) as a source of BMDC. We show in this study that IL-23 is required for optimal induction of IL-17-producing cells when APC are activated by mycobacteria. Specifically, the frequency of IL-17-producing CD4 T cells is significantly reduced in cultures with IL-23-deficient BMDC, compared with intact BMDC (Fig. 4*a*). To determine the ability of the TCRTg T cells to spontaneously make IL-17 in the absence of their peptide Ag, we omitted the peptide during the ELISPOT and show in this study that frequency of IL-17-producing cells in the absence of peptide is significantly reduced (Fig. 4*b*).

We hypothesize, therefore, that BCG induces TGF β and IL-6 production by the APC, and that this results in the expression of IL-23R on newly activated T cells (18). These T cells are then able to use the IL-23 produced by the APC in response to BCG (Fig. 3*b*). When IFN- γ is added to the mix either exogenously as in Fig. 4*a* or due to the expansion of IFN- γ -producing T cells (Fig. 1), then it will play two roles. First, it will increase the amount of IL-12p70 from BCG-infected APC (Fig. 3) and also up-regulate the IL-12R β 2 on newly activated T cells (18). In this way, the IFN- γ -producing T cells will expand and further limit the availability of IL-23 (Fig. 3*b*), thereby limiting the expansion of the IL-17-producing population.

In summary, we show that an IL-17-producing CD4 T cell population can be generated by mycobacterially activated APC and that IFN- γ regulates the generation of these cells. Our data lend further support to the hypothesis that IFN- γ is required to control IL-17-producing cells and thus the severe pathology that is seen in IFN- γ -deficient mice with experimental autoimmune encephalitis and collagen-induced arthritis or mycobac-

terial infection (15, 34–37). More specifically, an inability to produce sufficient IFN- γ to dampen IL-17-mediated inflammation during chronic infection may predispose individuals to inappropriate tissue-damaging responses. This inability may thus provide a basis for the association between mycobacterial exposure and arthritis (28, 29). Further, this work suggests a basis for the epidemiological data where decrease of infectious diseases correlates with the increase of certain inflammatory types of autoimmune diseases (38). Specifically, increased induction of IFN- γ -producing cells by pathogens may limit the production and accumulation of IL-17-producing cells in the periphery and thereby limit the potential for immune-mediated pathologic consequences for the host tissue.

Acknowledgments

We thank Drs. Anne O'Garra, Manuel T. Silva, João P. Pereira, and Rui Appelberg for helpful discussions and critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

- Arend, W. P. 2002. The balance between IL-1 and IL-1R α in disease. *Cytokine Growth Factor Rev.* 13: 323–340.
- Lawlor, K. E., I. K. Campbell, D. Metcalf, K. O'Donnell, A. van Nieuwenhuijze, A. W. Roberts, and I. P. Wicks. 2004. Critical role for granulocyte colony-stimulating factor in inflammatory arthritis. *Proc. Natl. Acad. Sci. USA* 101: 11398–11403.
- Steinman, R. M., and M. C. Nussenzweig. 2002. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA* 99: 351–358.
- Xanthoulea, S., M. Pasparakis, S. Kousteni, C. Brakebusch, D. Wallach, J. Bauer, H. Lassmann, and G. Kollias. 2004. Tumor necrosis factor (TNF) receptor shedding controls thresholds of innate immune activation that balance opposing TNF functions in infectious and inflammatory diseases. *J. Exp. Med.* 200: 367–376.
- Iwasaki, A., and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* 5: 987–984.
- Pasare, C., and R. Medzhitov. 2004. Toll-dependent control mechanisms of CD4 T cell activation. *Immunity* 21: 733–741.
- Pasare, C., and R. Medzhitov. 2005. Toll-like receptors: linking innate and adaptive immunity. *Adv. Exp. Med. Biol.* 560: 11–18.
- Sakaguchi, S. 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101: 455–458.
- O'Garra, A., and P. Vieira. 2004. Regulatory T cells and mechanisms of immune system control. *Nat. Med.* 10: 801–804.
- O'Garra, A., L. Steinman, and K. Gijbels. 1997. CD4⁺ T-cell subsets in autoimmunity. *Curr. Op. Immunol.* 9: 872–883.
- Cope, A. P. 1998. Regulation of autoimmunity by proinflammatory cytokines. *Curr. Op. Immunol.* 10: 669–664.
- Pearl, J. E., B. M. Saunders, S. Ehlers, I. M. Orme, and A. M. Cooper. 2001. Inflammation and lymphocyte activation during mycobacterial infection in the interferon- γ -deficient mouse. *Cell Immunol.* 211: 43–50.
- Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon- γ genes. *Science* 259: 1739–1742.
- Dalton, D. K., L. Haynes, C. Q. Chu, S. L. Swain, and S. Wittmer. 2000. Interferon- γ eliminates responding CD4 T cells during mycobacterial infection by inducing apoptosis of activated CD4 T cells. *J. Exp. Med.* 192: 117–122.
- Cooper, A. M., L. B. Adams, D. K. Dalton, R. Appelberg, and S. Ehlers. 2002. IFN- γ and NO in mycobacterial disease: new jobs for old hands. *Trends Microbiol.* 10: 221–226.
- Veldhoen, M., R. Hocking, C. Atkins, R. Locksley, and B. Stockinger. 2006. TGF- β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24: 179–189.
- Betтели, E., Y. Carrier, W. Gao, T. Korn, T. Strom, M. Oukka, H. Weiner, and V. Kuchroo. 2006. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. *Nature* 441: 235–238.
- Mangan, P., L. Harrington, D. O'Quinn, W. Helms, D. Bullard, C. Elson, R. Hatton, S. Wahl, T. Schoeb, and C. Weaver. 2006. Transforming growth factor- β induces development of the Th17 lineage. *Nature* 441: 231–234.
- Langrish, C., Y. Chen, W. Blumenschein, J. Mattson, B. Basham, J. Sedgwick, T. McClanahan, R. Kastelein, and D. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201: 233–240.
- Nakae, S., A. Nambu, K. Sudo, and Y. Iwakura. 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171: 6173–6177.
- Nakae, S., S. Saijo, R. Horai, K. Sudo, S. Mori, and Y. Iwakura. 2003. 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.

22. Fossiez, F., O. Djossou, P. Chomarat, L. Flores-Romo, S. Ait-Yahia, C. Maat, J. Pin, P. Garrone, E. Garcia, S. Saeland, et al. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* 183: 2593–2603.
23. Jones, C. E., and K. Chan. 2002. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene- α , and granulocyte-colony-stimulating factor by human airway epithelial cells. *Am. J. Resp. Cell Mol. Biol.* 26: 748–753.
24. Ye, P., F. H. Rodriguez, S. Kanaly, K. L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, et al. 2001. Requirement of interleukin-17 receptor signalling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J. Exp. Med.* 194: 519–527.
25. Park, H., Z. Li, X. Yang, S. H. Chang, R. Nurieva, Y. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin-17. *Nat. Immunol.* 6: 1133–1141.
26. Harrington, L. E., R. D. Hatton, P. R. Mangan, H. Turner, T. L. Murphy, K. M. Murphy, and C. T. Weaver. 2005. 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.
27. Khader, S., J. Pearl, K. Sakamoto, L. Gilmartin, G. Bell, D. Jelley-Gibbs, N. Ghilardi, F. deSavauge, and A. Cooper. 2005. 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.
28. Harrington, J. 1998. Mycobacterial and fungal arthritis. *Curr. Opin. Rheumatol.* 10: 335–334.
29. Moudgil, K. D. 1998. Diversification of response to hsp65 during the course of autoimmune arthritis is regulatory rather than pathogenic. *Immunol. Rev.* 164: 175–184.
30. Lutz, M. B., N. Kukutsch, A. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods* 223: 77–92.
31. Huygen, K., E. Lozes, B. Gilles, A. Drowart, K. Palfliet, F. Jurion, I. Roland, M. Art, M. Dufaux, J. Nyabenda, et al. 1994. Mapping of TH1 helper T-cell epitopes on major secreted mycobacterial antigen 85A in mice infected with live *Mycobacterium bovis* BCG. *Infect. Immun.* 62: 363–370.
32. Barnden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based α - and β -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76: 34–40.
33. Umemura, M., H. Nishimura, K. Hirose, T. Matsuguchi, and Y. Yoshikai. 2001. Overexpression of IL-15 in vivo enhances protection against *Mycobacterium bovis* bacillus Calmette-Guerin infection via augmentation of NK and T cytotoxic 1 responses. *J. Immunol.* 167: 946–956.
34. Ferber, I. A., S. Brocke, C. Taylor-Edwards, W. Ridgway, C. Dinisco, L. Steinman, D. Dalton, and C. Fathman. 1996. Mice with a disrupted IFN- γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol.* 156: 5–7.
35. Chu, C. Q., S. Wittmer, and D. Dalton. 2000. Failure to suppress the expansion of the activated CD4 T cell population in IFN- γ -deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 192: 123–128.
36. Vermeire, K., H. Heremans, M. Vandeputte, S. Huang, A. Billiau, and P. Matthys. 1997. Accelerated collagen-induced arthritis in IFN- γ receptor-deficient mice. *J. Immunol.* 158: 5507–5513.
37. Matthys, P., K. Vermeire, H. Heremans, and A. Billiau. 2000. The protective effect of IFN- γ in experimental autoimmune diseases: a central role of mycobacterial adjuvant-induced myelopoiesis. *J. Leukocyte Biol.* 68: 447–454.
38. Bach, J. F. 2002. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347: 911–920.