Glucocorticoid-resistant Th17 cells are selectively attenuated by cyclosporine A

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Glucocorticoids remain the cornerstone of treatment for inflammatory conditions, but their utility is limited by a plethora of side effects. One of the key goals of immunotherapy across medical disciplines is to minimize patients’ glucocorticoid use. Increasing evidence suggests that variations in the adaptive immune response play a critical role in defining the dose of glucocorticoids required to control an individual’s disease, and Th17 cells are strong candidate drivers for nonresponsiveness (also called steroid resistance [SR]). Here we use gene-expression profiling to further characterize the SR phenotype in T cells and show that Th17 cells generated from both SR and steroid-sensitive individuals exhibit restricted genome-wide responses to glucocorticoids in vitro, and that this is independent of glucocorticoid receptor translocation or isoform expression. In addition, we demonstrate, both in transgenic murine T cells in vitro and in an in vivo murine model of autoimmune arthritis, that Th17 cells are reciprocally sensitive to suppression with the calcineurin inhibitor, cyclosporine A. This result was replicated in human Th17 cells in vitro, which were found to have a conversely large genome-wide shift in response to cyclosporine A. These observations suggest that the clinical efficacy of cyclosporine A in the treatment of SR diseases may be because of its selective attenuation of Th17 cells, and also that novel therapeutic strategies that target either Th17 cells themselves or the effector memory T-cell population from which they are derived, would be strong candidates for drug development in the context of SR inflammation.

Th17 | glucocorticoid | steroid resistance | calcineurin inhibition | uveitis

Glucocorticoids are the single most commonly used drug to treat all inflammatory diseases in man (1). However, their benefits are balanced against a plethora of side effects, including centripetal obesity, diabetes mellitus, hypertension, and osteoporosis, resulting in a substantial iatrogenic burden of ill health and reduced life expectancy (2). This finding has particular relevance to so-called steroid-resistant (SR) individuals, who require intolerable doses of glucocorticoids to maintain disease remission, and account for up to a third of patients with inflammatory diseases across all medical specialties (3). Better understanding of the biological mechanisms that underlie the SR phenotype therefore promises to reveal novel strategies to minimize glucocorticoid use in clinical practice.

The effects of glucocorticoids are mediated through the glucocorticoid receptor (GR), which regulates a broad spectrum of physiological processes and genetic mutations and polymorphisms of the GR gene (NR3C1) give rise to well-described rare SR syndromes (4). However, in the absence of such a premorbid abnormality in steroid signaling there is no unifying explanation of the SR phenotype. Candidate mechanisms include overexpression of the inhibitory β-isofrom of the GR (GR-β) (1, 5), but there is also increasing evidence to suggest that specific variations in the adaptive immune response to glucocorticoid therapy play a key role (6–8).

Th17 cells represent a distinct CD4+ lineage defined by the secretion of characteristic cytokines, in particular IL-17A (hereafter referred to as IL-17), IL-17F, and IL-22 (9). In humans, Th17 cells are subsets of effector memory T-helper cells that express the chemokine receptor CCR6 (10), and they play a vital role in host defense against intracellular bacterial and fungal infections (9). These cells are also key drivers in the development of autoimmune conditions (11). Increasing evidence supports the concept of a SR Th17 phenotype, and their adoptive transfer, or the overexpression of their canonical transcription factor retinoid-related orphan receptor (ROR)–γt, has been shown to induce SR disease in a murine model of asthma (12, 13). In humans, glucocorticoid-resistant Th17 cells express the multidrug resistance type 1 protein, which is inhibited by cyclosporine A (CsA) (14), and historically CsA was one of the first drugs used to successfully rescue SR diseases (1). We therefore hypothesized that CsA would selectively inhibit human Th17 cells.

In this article, we compare the effects of glucocorticoids and CsA on human and murine Th17 cells. First, we quantified the in

Significance

Cyclosporine A was one of the first drugs used in clinical practice to successfully rescue glucocorticoid-resistant inflammatory diseases. In this article we extend the characterization of glucocorticoid-resistant human Th17 cells, and demonstrate that this effector memory T-cell subset is reciprocally attenuated by cyclosporine A. This therapeutic paradigm was confirmed in a murine model of autoimmune arthritis, refining our understanding of cyclosporine A’s effect on the adaptive immune response. These data support the rationale for Th17-targeting therapies in the treatment of glucocorticoid-resistant inflammation.

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vitro expression of IL-17 in effector memory CD4+ T cells from the peripheral blood of patients with an autoimmune disease (uveitis) for which the SR phenotype represents a significant clinical challenge (7), and compared this with controls. Second, we characterized the effect of glucocorticoids on the gene-expression profile of human Th17 cells in vitro and examined whether this was affected by differential GR nuclear translocation or isomorph expression. Third, we interrogated the SR Th17 paradigm in enriched murine transgenic Th17 cells in vitro, and also in vivo using an allied mouse model of organ-specific autoimmune, to test the hypothesis that the calcineurin inhibitor CsA would have a reciprocal effect to glucocorticoids on IL-17 expression. Fourth, we assessed the effects of CsA on human Th17 cells to see if the results seen in mouse were replicated in man. These data show that Th17 cells are refractory to glucocorticoid suppression at a genome-wide level in a GR-independent manner and are conversely sensitive to inhibition with CsA.

**Results**

**Human Th17 Cells Exhibit a Restricted Genome-Wide Response to Glucocorticoids.** To profile the glucocorticoid response of Th17 cells (Fig. 1 A and B), memory CD4+CCR6+ cells were sorted from the peripheral blood of independent donors and cultured for 14 d in the presence of polarizing cytokines, whereas control CD4+CCR6− T cells were simultaneously sorted and cultured in the absence of polarizing cytokines (10). As shown in Fig. S1A, we did not find a difference in the proportion of CD4+ cells expressing IL-17 in either culture condition from steroid-sensitive (SS) patients, SR patients, or healthy control (HC) donors. Total IL-17 production was 39.1% from CCR6+ and CCR6− cells following 14-d culture in Th17 or control (Th0) conditions. Representative FACS plots from HC, SS patients, and SR patients are shown. (B) Percentage (±SEM) of intracellular IL-17 and IFN-γ single-positive cells and IL-17/IFN-γ double-positive or double-negative cells in CD4+CCR6+ and CD4+CCR6− cells following 14-d culture in Th17 or control (Th0) conditions, calculated according to the gates shown in A (n = 25). The data for HC, SS, and SR donors has been compiled as there was no difference in the phenotype of cells across these groups (Fig. S1A). (C) Hierarchical clustering analysis of SS and SR Th17 and Th0 cells (untreated and Dex-treated). Up-regulated genes are shown in red on the heat map and down-regulated genes are in blue. The IL-17A and IL-17F expression in each culture is highlighted. (D) PCA of genes with at least twofold changes between any two of the four conditions. The shift in gene expression in response to Dex is proportional to the length of the arrow. These are presented as untreated and Dex treated Th17 and Th0 cells derived from SS or SR patients; D, post-Dex treatment.

We then analyzed the genome-wide expression profiles in Th17 cells versus Th0 cells treated with or without the synthetic glucocorticoid dexamethasone (Dex) using Affymetrix U133 2.0 GeneChips. A total of 24 samples were analyzed (triplicates for each patient group and in vitro condition), and Dex was used at 1 × 10−6 M (Fig. S1B). We found that the expression of classic Dex-inducible genes, such as FKBP5 and TSC22D3, were elevated in both Th17 and Th0 cells. Hierarchical clustering analysis confirmed the up-regulation of IL-17A and IL-17F in human Th17 cultures (Fig. 1C). However, principal component analysis (PCA) of all of the differentially expressed genes (defined as a twofold change with a P value <0.05) revealed that the genome-wide expression changes induced by Dex treatment in Th17 cells from both SS and SR individuals were significantly restricted compared with Th0 cells (Fig. 1D). Analysis of the combined dataset confirmed that, overall, human Th17 cells are less responsive to glucocorticoids than T0 cells (Fig. S1C). This finding was also evident in terms of T-cell proliferation (Fig. S1D). Furthermore, we also observed that the total number of genes responding to Dex in human Th17 cells was much less than in Th0 cells (Fig. S1C and Table S1). In particular, the expression of nuclear factor κ light polypeptide gene enhancer in B-cells inhibitor ζ (NFKBIZ), which promotes IL-17 expression (15), was suppressed in Th0 cells, whereas its expression was increased by Dex in Th17 cells.

**The Failure of Human Th17 Cells to Respond to Glucocorticoids Is GR-Independent.** Glucocorticoid-induced nuclear translocation of the GR is the first step in the process of transactivation of genes containing glucocorticoid-response elements (16). To determine whether a defect in GR nuclear translocation could explain the glucocorticoid refractory nature of human Th17 cells, we examined the subcellular localization of GR in Th17 cells using confocal microscopy. Immunofluorescent staining showed that GR, which was primarily cytoplasmic in resting cells, translocated to the nucleus following Dex treatment (Fig. 2A and Movies S1–S4). The nuclear fluorescent intensity of GR was also...
assessed in control Th0 cells and indicated that GR translocation is not significantly perturbed in human Th17 cells (Fig. 2B). Overexpression of the functionally inactive GR-β isoform could also be responsible for glucocorticoid resistance (5), and we therefore interrogated the expression of total GR and GR-β. We did not find a significant difference in the expression of GR or GR-β between human Th17 and Th0 cells, and this was not altered by the addition of Dex (Fig. 2C and D). These results suggest that the failure of human Th17 cells to respond to glucocorticoids was not because of differences in total GR expression, GR nuclear translocation, or overexpression of the dominant-negative GR-β isoform.

Murine Th17 Cells Are SR and Are Preferentially Suppressed with CsA.

Given the success of CsA in clinically rescuing SR inflammation (1) and that Th17 cells are resistant to Dex, we hypothesized that Th17 cells are susceptible to CsA inhibition. To test this, we first used a murine in vitro system to generate more highly enriched populations of IL-17- and IFN-γ-expressing CD4+ T cells to interrogate the comparative effect of Dex (Fig. S2) and CsA on these two canonical T-cell subsets. Using naïve CD4+ cells from hen egg lysozyme (HEL)-specific T-cell receptor (TCR) transgenic mice on a B10.BR background (3A9), we generated T cells that were highly enriched for the expression of IL-17 ("Th17"), and control cells that were highly enriched for the expression of IFN-γ ("Th1") (Fig. S3A). Consistent with our human data, murine Th17 cells continued to proliferate despite the presence of Dex at a concentration that fully suppressed Th1 cells (Fig. 3B). Conversely, the reverse was seen in CsA-treated cultures, in which the division of Th17 cells was fully suppressed at a dose that was unable to inhibit Th1 cells (Fig. 3B). These observations were then replicated, first using CD4+ cells from ovalbumin (OVA)-specific OT-II mice (on a C57BL/6 background), confirming that this was not a phenomenon limited to a particular TCR transgenic strain (Fig. 3C and D), and second with an alternative calcineurin inhibitor, tacrolimus (Tac) (Fig. S3). Hence, Dex and calcineurin inhibition with either CsA or Tac had reciprocal effects on Th1 and Th17 cell proliferation, with Th17 cells being less responsive to glucocorticoids, and more responsive to calcineurin inhibition, than Th1 cells.

To examine whether Dex and CsA had reciprocal effects on Th1 and Th17 cell proliferation in vivo, we used the organ-specific model of Th1/Th17-driven inflammation, experimental autoimmune uveitis (EAU) (17). For comparison of the effect of CsA and Dex on T-cell subsets, drug concentrations were titrated to establish the minimum dose at which equivalent suppression of inflammation was achieved, as measured by direct visualization of the organ-specific immune response in the eye using topical endoscopic fundal imaging (TEFI) (Fig. S4A) (18). We then treated EAU mice with either Dex or CsA and evaluated their effects by a combination of TEFI and histology (Fig. 4A–D). This finding demonstrated a significant reduction of disease severity with both drugs following the first and subsequent treatments. Furthermore, there was a significant and equal reduction of the total number of organ-infiltrating CD4+ T cells in both Dex and CsA treated mice, and the proportion of infiltrated Ly6G+ neutrophils and CD11b+ myeloid cells was also similarly reduced in both treatment groups (Fig. 4E). However, despite achieving equivalent suppression of CD4+ T-cell numbers with each drug, the IL-17 and IFN-γ cytokine profiles of tissue infiltrating CD4+ cells was strikingly different. Dex suppressed IFN-γ expression by 45%, whereas the proportion of IL-17–expressing cells was relatively increased. In contrast, CsA almost completely ablated IL-17 expression with a 91% reduction in the proportion of cells expressing this cytokine compared with control mice. IFN-γ expression was also significantly reduced by CsA (Fig. 4F). This drug-specific cytokine skewing was particularly evident when the ratio of IL-17/IFN-γ was calculated. Dex decreased IFN-γ more than IL-17, and CsA—which decreased both cytokines—inhibited IL-17 expression more than IFN-γ (Fig. S4B). Consistent with this finding, mRNA expression of the Th17 associated cytokines Il17a, Il17f, and Il22 in ocular-infiltrating CD4+ T cells from Dex-treated mice was increased compared with the infiltrating CD4+ T cells from control.

Fig. 2. GR expression in human Th17 cells. (A) GR expression in CD4+CCR6+ and CD4+CCR6− cells following 14-d culture in Th17 or control (Th0) conditions. Cells were treated for 30 min with Dex. Con, Control. A representative of five independent experiments is shown. (Magnification: 1,260×.) (B) Quantitative image analysis of GR nuclear density in control and Dex-treated cells. Data represented as mean ± SEM (n = 5; 163 ± 26.16 cells analyzed per experiment over five fields of view). (C) Real-time PCR analysis for total GR in Th17 and Th0 cells cultured for 14 d and then treated with or without Dex for 30 min (n = 5). Data represented as mean ± SEM. (D) Relative expression of the GR-β isoform with or without Dex treatment for 30 min (n = 5). Data represented as mean ± SEM.
Untreated animals. Moreover, only CsA treatment significantly reduced the expression of Th17- and Th1-specific transcription factors, Rorc, Tbx21, and Ahr, quantified by PCR (Fig. 4G). Therefore, these data demonstrate that Th17 cells escape Dex suppression in vivo, whereas calcineurin inhibition controls both Th17- and Th1-mediated inflammation in this organ-specific model of autoimmunity.

**Human Th17 Cells Are Also Exquisitely Sensitive to Calcineurin Inhibition.**

To determine whether the dominant anti-Th17 effects of CsA seen in mice would be replicated in man, human Th17 and Th0 cells generated using identical conditions to the glucocorticoid experiments presented in Fig. 1 were treated with CsA for 24 h. This process suppressed the expression of both IL-17 and IFN-γ in human Th17 cells (Fig. 5A). However, as seen in murine CD4+ T cells, IL-17 expression was suppressed by CsA to a greater extent than IFN-γ (Fig. 5B). This suppression of Th17 cells by calcineurin inhibition was replicated using Tac (Fig. S5), and further reflected in reduced expression of the key Th17 cell transcription factor RORC, with concomitant ablation of IL17A mRNA, and an over 90% reduction in IL17F. This result was despite the continued expression of AHR. Conversely, the expression of IFN-γ (IFNG) was only reduced by 43%, and expression of the key transcription factor for Th1 differentiation Tbx21 was not changed in CsA-treated cells (Fig. 5C).

RNA-seq technology (Illumina) was used for gene-expression profiling of human Th17 and Th0 cells to assess genome-wide changes in response to CsA treatment. PCA of all genes with at least a twofold difference in their expression between any two conditions showed that significant changes in gene expression were induced in human Th17 cells following CsA treatment, but only a small expression pattern change was seen in Th0 cells (Fig. 5D). Importantly, 210 of 246 genes uniquely expressed in Th17 cells were suppressed by CsA, including IL17A, IL22, IL26, IL23R, and CD161 (Fig. S6 and Table S2), whereas the expression of only 2% of all Th0 specific genes was changed by CsA.
Treatment (Fig. S6B). These data demonstrate that CsA results in suppression of the human Th17 transcriptome at a concentration that had a comparatively limited effect on control Th0 cells.

Discussion

In this study we have extended previous reports of attenuated Th17 cell responses to glucocorticoids, demonstrating that this is exhibited at a genome-wide level in human CD4+ T cells and is independent of GR isoform expression and nuclear translocation. In addition, we have shown in both mouse and man that there is a reciprocal sensitivity of Th17 cells to calcineurin inhibition with CsA. These observations are consistent with the clinical efficacy of CsA in the treatment of SR conditions, suggesting that one of its key effects on the adaptive immune response is to selectively suppress Th17 cells that escape glucocorticoid inhibition.

The SR Th17 paradigm has already been described in terms of restricted suppression of cell proliferation in murine in vitro and in vivo systems in comparison with IL-4, IL-5, and IL-13 expressing Th2 cells (12). This result has also recently been replicated in human in vitro cultures in comparison with IFN-γ-expressing cells (14). We therefore examined whether effector memory CD4+CCR6+ T cells from patients with clinically defined SR disease were biased to express IL-17 when exposed to the Th17 cell-promoting cytokines IL-1β, IL-23, and IL-6, and found that there was no difference between SR and SS patients, or HCs in terms of IL-17 expression (Fig. 1A and Fig. S1A). This finding suggests that effector memory cells from SR patients have no greater propensity to produce IL-17 than those from SS individuals when cultured under Th17 polarizing conditions. Th17 cells, as a subtype of effector T cells, are characterized by the expression of not only IL17A, but also a set of genes encoding cytokines, chemokines, and key signaling molecules promoting inflammation and antimicrobial immunity. Therefore, at a genome-wide level, our PCA analysis of microarray data demonstrated that restriction of the response to glucocorticoid treatment was attributable to the T-cell lineage (i.e., Th17 vs. control Th0 cells) rather than whether the T-cell donor was SS or SR. This finding suggests that beyond cytokine production, Th17 cells have intrinsic factors that are hyporesponsive to glucocorticoid treatment. For example, NKFBIZ is essential for Th17 cell polarization and directly regulates IL17 expression by binding to its promoter in mouse (19). We found it was induced in Th17 cells by Dex (Table S1). Therefore, the persistence of NKFBIZ in Dex-treated human Th17 cells (Table S1) could be key to their maintenance of RORC expression (20) and may also interfere with GR function (21, 22). In addition, recent reports of genome-wide binding profiles have demonstrated the transcription factors NF-κB and Stat3, both of which are activated in Th17 cells, may antagonize GR functions by changing the DNA binding sites of GR (23). Furthermore, it is possible that altered GR binding affinity at glucocorticoid response elements plays a role (19).

CsA’s effect on IL-17– and IFN-γ–expressing cells is strikingly opposite to that of glucocorticoids; it was shown to selectively suppress Th17 more than Th1 cell proliferation in vitro using different types of murine CD4+ T cells (with two transgenic TCRs: HEL- and OVA-specific) (Fig. 3). Furthermore, in an in vivo model of organ-specific autoimmunity, which is driven by both Th1 and Th17 cells (24), IL17a, IL17f, and IL22 continued to be expressed in Dex-treated animals, despite the total cell number being markedly reduced compared with control animals. Conversely, there was complete ablation of the expression of IL-17, IFN-γ, and the Th1- and Th17-associated transcription factors Rorc, Ahr, and Tbx21 in residual tissue-infiltrating CD4+ T cells following CsA treatment. This finding confirms that depletion of either Th1 or Th17 cells can lead to clinical ablation of human murine intraocular inflammation (24). However, the complete ablation of CD4+ T-cell IL-17 expression by chronic calcineurin inhibitor treatment could also have deleterious long-term effects at sites of inflammation, given the important role of IL-17 in tissue repair (25).

The clear bias CsA exhibited toward the suppression of IL-17 more than IFN-γ (Fig. 5B) was further reflected in the complete inhibition of IL17A mRNA and profound reduction of IL17F and RORC expression in CsA-treated human Th17 cells, whereas the expression of the Th1 transcription factor TBX21 was maintained with less than 50% reduction in IFNG by CsA in human Th17 cells (Fig. 5C). Furthermore, our RNA-seq data demonstrated that the genome-wide response to CsA treatment of human IFN-γ–expressing Th0 cells compared with Th17 cells is greatly restricted and the previously identified human Th1 signature genes, including IL22, IL26, IL17A, IL23R (26), and CD161 (27), were among the top six genes that were highly suppressed by CsA treatment (Fig. S6 and Table S2). This finding is again opposite to the effect of Dex on the gene-expression profile of these cells (Fig. 5D and Fig. S1C), suggesting the difference of CsA responses between Th17 and Th0 cells is also across the whole transcriptome, as well as on signature cytokines and cell-surface markers for human Th17 cells.

Although others have previously shown that when CsA is used to treat inflammation, it reduces IL-17 concentrations in the serum of patients, and also in mixed cultures of cells in vitro...
(28, 29), this is, to our knowledge, the first time this T-helper cell subtype-specific effect has been demonstrated. Similar suppression of IL-17 expression and cell division in Th17 cells treated with Tac also suggests that this effect is common across calcineurin inhibitors. Furthermore, our affirmation of the human Th17 SR phenotype makes the presence of these cells, either in peripheral blood or inflamed tissue, a candidate biomarker for the SR disease. In addition, our data suggest that one of the principal actions of CsA is its selective attenuation of IL-17–secreting CD4+ T cells, which is supported by its efficacy in the treatment of Th17-associated disorders, such as inflammatory bowel disease and psoriasis (30, 31), and this is likely to have effects beyond the inhibition of IL-17 alone. Hence, alternative approaches to treatment that have minimal effects on nonimmune tissues and exquisitely interfere with Th17 cell function, either by targeting Th17 cells themselves or the effector memory CD4+ T cell population from which they are derived, would be strong candidates for drug development in the context of SR inflammation.

Methods

Patient and Healthy Control Details. Peripheral blood mononuclear cells were obtained from up to 100 mL of blood from HC and patients following informed consent in accordance with Institutional Review Board-approved protocols at the NIH (08-EI-0099), and National Health Service Research Ethics Committee-approved protocols at University Hospitals Bristol (National Health Service) Foundation Trust, United Kingdom (04/Q02002/84). Further details are described in SI Methods, as are further details for all of the techniques listed below.

EAU Induction, Treatment, Clinical Assessment, and Organ Desection. B10.RIII mice were immunized for EAU induction. Dex was administered subcutaneously and CsA was administered by oral gavage. TEFI was used to assess the clinical score of EAU (Table S3) (18). A single-cell suspension of tissues was isolated using a tissue dissociation method.

Cell Culture and T Cell Proliferation Assays. Murine Th1 and Th17 cultures were cultured, as previously reported (32). Human peripheral CD4(+)CCR6(+) and CD4(+)CCR8(+) cells were cultured in IL-2 alone (Th0) or with Th17 polarizing cytokines (Th17). T-cell proliferation was measured by pulsing with 37 kBq [3H]thymidine (Perkin-Elmer) per well for the final 12–16 h of cultures.

Flow Cytometry and FACS Sorting. All flow cytometry experiments were conducted on the BD LSR II and FACS sorting was carried out using the BD Influx system.

Quantitative Real-time PCR. Total RNA was extracted and real-time PCR assays were performed using Taqman reagents (Applied Biosystems).

Immunofluorescence. GR trafficking into the nucleus was optimized and quantified using a Leica SP5 confocal imaging system.

Affymetrix Microarray Data Collection, Analysis, and RNA-seq. RNA was hybridized to GeneChip U133 plus 2.0 arrays (Affymetrix) according to the manufacturer’s protocols. RNA-seq libraries were sequenced with paired-end 50-bp reads on an Illumina HiSeq. 2000.

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1. Barnes PJ, Adcock IM (2009) Glucocorticoid resistance in inflammatory diseases. Lancet 373(9678):1905–1917.
2. Fan H, Morand EF (2012) Targeting the side effects of steroid therapy in autoimmune disease: The role of GIIL. Discov Med 13(69):123–133.
3. Schwetz LP, Lee RW, Dayan CM, Dick AD (2009) Glucocorticoids and the emerging importance of T cell subsets in steroid resistant diabetes. Immunopharmacol Immunotoxicol 31(1):1–22.
4. Miranda TB, Morris SA, Hager GL (2013) Complex genomic interactions in the dynamic transcriptional regulation of the glucocorticoid receptor. Mol Cell Endocrinol 380(1-2):16–24.
5. Webster JC, Oakley RH, Jewell CM, Cidlowski JA (2001) Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: A mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci USA 98(12):6865–6870.
6. Lee RW, et al. (2007) CD4+CD25(+) T cells in inflammatory diseases refractory to treatment with glucocorticoids. J Immunol 178(11):7941–7948.
7. Lee RW, Schwetz LP, Nicholson LB, Dayan CM, Dick AD (2009) Steroid refractory CD4+ T cells in patients with sight-threatening uveitis. Invest Ophthalmol Vis Sci 50(9):4273–4278.
8. Nanzer AM, et al. (2013) Enhanced production of IL-17A in patients with severe asthma is inhibited by 1alpha,25-dihydroxyvitamin D3 in a glucocorticoid-independent fashion. J Allergy Clin Immunol 132(2):297–304.
9. Basu R, Hatton RD, Weaver CT (2013) The Th17 family: Flexibility follows function. J Exp Med 210(3):427–439.
10. Barnes PJ, Adcock IM (2009) Glucocorticoid resistance in inflammatory diseases. J Allergy Clin Immunol 123(2):474–486.
11. Rosas B, Hatton RD, Weaver CT (2013) Transcription factors GATA-3 and RORα/γ determine the phenotype of allergic airway inflammation in a murine model of asthma. J Immunol 189(1):406–415.
12. Miranda TB, Morris SA, Hager GL (2013) Complex genomic interactions in the dynamic transcriptional regulation of the glucocorticoid receptor. Mol Cell Endocrinol 380(1-2):16–24.
13. Acosta-Rodriguez EV, et al. (2007) Surface phenotype and antigenic specificity of the human T cell receptor αβ: A role for co-receptor modifications. Biochim Biophys Acta 1779(1):165–65.
14. Scheinman RI, Cogswell PC, LoFrook AS, Baldwin JS, Jr (1995) Role of transcriptional activation of the IL-18 gene in mediation of immune suppression by glucocorticoids. Science 270(5243):283–286.
15. Langlais D, Couture C, Balabolke A, Drunin J (2012) The Stat3/GR interaction code: Predictive value of directindirect DNA nucleation for transcription outcome. Mol Cell 47(1):38–49.
16. Reger J, et al. (2006) Either a Th1 or a Th17 effector response can drive autoimmune-nity: Conditions of disease induction affect dominant effector category. J Exp Med 205(4):799–810.
17. Chen Y, Zhong M, Liang L, Gu F, Peng H (2014) Interleukin-17 induces angiogenesis in human choroidal endothelial cells in vitro. Invest Ophthalmol Vis Sci 55(10):6968–6975.
18. Wilson NJ, et al. (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 8(9):950–957.
19. Cosmi L, et al. (2008) Human interleukin 17 producing cells originate from a CD161+CD4+ T cell precursor. J Exp Med 205(8):1903–1916.
20. Forre O, Norwegian Arthritis Study Group. (1994) Radiologic evidence of disease modification in rheumatoid arthritis patients treated with cyclosporine. Results of a 48-week multicenter study comparing low-dose cyclosporine with placebo. Arthritis Rheum 37(10):1506–1512.
21. Vitale AT, Rodriguez A, Foster CS (1996) Low-dose cyclosporin A therapy in treating chronic, noninfectious uveitis. Ophthalmology 103(3):365–373; discussion 373–364.
22. Martin DA, et al. (2013) The emerging role of IL-17 in the pathogenesis of psoriasis: Preclinical and clinical findings. J Invest Dermatol 133(1):17–26.
23. Xavier RJ, Podolsky DK (2007) Unraveling the pathogenesis of inflammatory bowel disease. Nature 448(7152):427–434.
24. Shi G, et al. (2008) Phenotype switching by inflammation-inducing polarized Th17 cells, but not by Th1 cells. J Immunol 181(5):7025–7031.