Glucocorticoid therapy of antigen-induced arthritis depends on the dimerized glucocorticoid receptor in T cells

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Despite several side effects, glucocorticoids (GCs) have been widely used for 60 y to treat rheumatoid arthritis on the basis of their antiinflammatory effects. However, the cells targeted by GCs and the transcriptional mechanisms underlying their actions through the glucocorticoid receptor (GR) in steroid therapy remain poorly defined. Using cell type-specific GR-deficient mice subjected to antigen-induced arthritis (AIA) as a model of human rheumatoid arthritis, we show that GC action on T cells but not myeloid cells is critical for therapeutic intervention in AIA. Furthermore, the resistance of mice expressing a DNA binding-defective GR (GR^{dim}) to GC treatment reveals that dimerization of the GR is indispensable for the antiinflammatory effects. In these mice, the GC-induced suppression of T_H1 and T_H17 cell-derived proinflammatory cytokines is impaired. Our finding that IL-17A$^{-/-}$ mice are resistant to GC therapy, whereas IFN-$\gamma^{-/-}$ mice respond as efficiently as WT mice implies that IL-17–producing T cells and not IFN-γ–producing T cells are the most important targets for an efficient GC therapy. The present study's identification of the critical cell type and the mode of GR action in steroid therapy of AIA significantly advances our understanding of steroid therapy and should lead to therapies with greater efficiency and fewer side effects.

conditional knockout mice | activated T cells | corticosteroid therapy | chronic inflammation

Rheumatoid arthritis (RA) is a severe autoimmune disease characterized by massive inflammation of peripheral joints that subsequently leads to the progressive destruction of articular cartilage and bone (1). For more than 60 y, RA patients have been treated with glucocorticoids (GCs) (2) because of their unsurpassed antiinflammatory effects. Indeed, GCs remain an essential component of RA therapy (3) despite their severe side effects.

The glucocorticoid receptor (GR) is a nuclear receptor that resides in the cytoplasm in the absence of ligand. Upon hormone binding, the GR can interfere with signal transduction components in the cytoplasm, such as Jun N-terminal kinases or PI3 kinases. Nonetheless, the majority of GR molecules translocate into the nucleus (4), where they alter gene expression by acting as a transcription factor via two different modes of action: the GR can dimerize and bind to palindromic elements in the promoter of GC-regulated genes, or it can interact as a monomer with DNA-bound transcription factors such as NF-κB, activator protein 1 (AP-1), interferon regulatory factor 3 (IRF-3), and signal transducer and activator of transcription (STAT)-5 (5, 6).

Very few studies have addressed the mode of action required for GC antiinflammatory activities in animal inflammatory models (4, 7). GR^{dim} mice carry a point mutation in the DNA-binding domain, thereby abrogating dimerization and the DNA-binding capacity of the GR but leaving the interaction with NF-κB and AP-1 intact (7). In steroid therapy of phorbol ester-induced irritative inflammation, dimerization of the GR is not required (8). However, in contact hypersensitivity, dimerization and binding of the GR to DNA are necessary for the antiinflammatory effects (9). The cell type required to execute the antiinflammatory activities also differs in various inflammatory models. For example, myeloid cells are the target cells for the antiinflammatory effects of GCs in contact hypersensitivity (9) and septic shock models (10), whereas peripheral T cells are the primary targets in experimental autoimmune encephalomyelitis (a rodent model of multiple sclerosis) (11). Thus, the critical cell type and mode of GR action involved in steroid therapy seem to depend on the type of inflammation.

To identify the target cell types and mechanisms of GCs in RA therapy, we used the antigen-induced arthritis (AIA) mouse model. AIA is characterized by severe inflammation in knee joints, and the pathomechanism critically depends on T cells (12); in particular, $CD4^+$ T_H1 (13) and IL-17–producing $CD4^+$ cells (T_H17 cells) have been shown to be crucial (14, 15). However, the AIA model is also characterized by a strong infiltration of neutrophils, macrophages, and dendritic cells (DCs) into the knee joint cavity. Finally, AIA proceeds to a chronic phase with hyperplasia of the synovial lining, pannus formation, infiltration of mononuclear cells, and subsequently severe destruction of cartilage and bone. Here we demonstrate that the GR in T cells, but not in myeloid cells, is critical for suppression of inflammation by GCs and that GR dimer-dependent gene regulation is pivotal for the antiinflammatory effects of GCs in T cells in AIA and, at least in part, in another RA model.

Results

GCs Inhibit the Acute Phase of AIA. First, we established a therapeutic regimen of treating AIA with GCs (*Materials and Methods* and Fig. 1*A*). Treatment with the GR agonist dexamethasone (Dex; 1.25 mg/kg i.v.) resulted in reduced knee joint swelling compared with control-treated animals (Fig. 1*B*). The antiinflammatory effects of this GC dose were confirmed by histopathological analysis of knee joints 1 d after arthritis induction, the peak of the inflammatory response (Fig. 1*C*). In contrast

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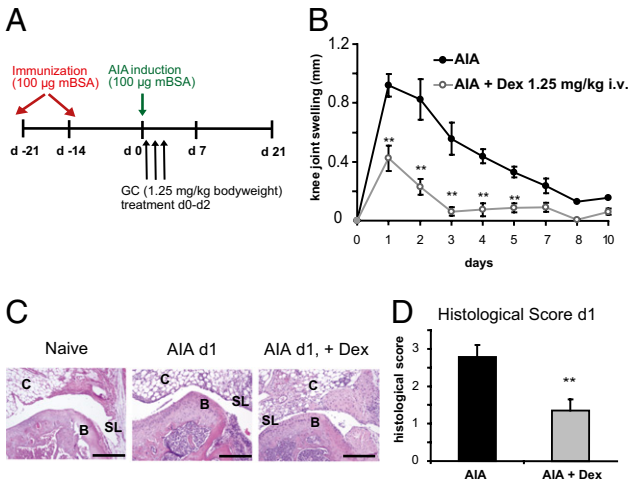

Fig. 1. GC treatment suppresses AIA. (*A*) Treatment scheme of AIA induction and Dex application (*Materials and Methods*). (*B*) Effect of Dex treatment on AIA knee joint swelling at indicated time points. (*C*) Representative H&E stainings of naïve healthy knee joints and of arthritic PBS- and Dex-treated joints at day 1. B, bone of joint; C, connective tissue; SL, synovial layer. (Scale bars, 0.2 mm.) (*D*) Histological score of PBS- and Dex-treated arthritic knee joints at day 1 according to Tolk and Földi's grading of joint inflammation (*Materials and Methods*). In *B* and *D*, $n = 8$; $^{**}P < 0.01$.

to healthy naïve mice, knees from mice with AIA exhibited hyperplasia of synovial cells, strong exudate, and massive infiltration of polymorphonuclear cells in the arthritic joints (Fig. 1*C*), resembling the histopathological findings in human RA (16, 17). Importantly, in joints of Dex-treated animals, cellular infiltration in the articular and connective tissue and synovial inflammation were reduced (Fig. 1*C*), resulting in a significantly lower histopathological score (Fig. 1*D*). Thus, our protocol for treatment of AIA with Dex resembles the therapeutic effects of GCs in the acute phase of human RA and so represents a suitable model to investigate the relevant cell type involved in steroid therapy.

Immune Suppressive Actions of GCs Require the GR in T Cells. We first studied $GR^{LysMCre}$ mice lacking the GR in myeloid cells (9) because neutrophils and macrophages are early joint-infiltrating cells in the development of AIA. AIA in $GR^{LysMCre}$ mice resulted in a massive knee joint swelling comparable to that in GR^{flox} control mice (Fig. 2*A*). Disease progression in the mutant animals was similar to that in GR^{flox} mice, excluding a role of endogenous GCs in macrophages. Furthermore, AIA could be efficiently repressed by GC treatment in both genotypes (Fig. 2*A*). Thus, the GR in myeloid cells is not essential for the anti-inflammatory effects of GCs in AIA.

Because of the involvement of DCs in antigen presentation and their ability to produce cytokines, we analyzed $GR^{CD11cCre}$ mice. Generated by crossing GR^{flox} mice with CD11cCre mice (18), the GR-encoding gene is almost completely ablated in $CD11c^{+}$ DCs and partially, but not completely, in macrophages and T cells (Fig. S1 *A* and *B*). Both $GR^{CD11cCre}$ and GR^{flox} mice developed severe knee joint swelling, and Dex could effectively suppress the symptoms of AIA (Fig. 2*B*), indicating that the GR in DCs is not critical for immune suppression of AIA.

Next we examined $GR^{CD19Cre}$ mice with a conditional deletion of the GR in B cells (Fig. S1 *C* and *D*) (19). $GR^{CD19Cre}$ mice showed a disease course similar to that in GR^{flox} mice, and the clinical signs could be as efficiently suppressed by GC treatment as in control animals (Fig. 2*C*). This is in line with IgG serum analysis data from arthritic mice upon GC treatment, whereby Dex had no influence on the anti-mBSA (methylated BSA) IgG level (Fig. S2*A*).

Because AIA is a T cell-dependent inflammatory disease (20), we investigated the capacity of GCs to suppress AIA in GR^{LckCre} mice. These mice display a very efficient recombination of the GR loxP allele in double- and single-positive thymocytes as well as in mature T cells (indicated by real-time PCR) (Fig. S1 *E* and *F*), and deletion of the GR does not affect their abundance (Fig. S3). Although the inflammatory response in the absence of GCs was not altered in GR^{LckCre} mice, suppression of the inflammatory swelling response after Dex treatment was severely impaired (Fig. 2*D*). GCs also failed to reduce cellular infiltrates in knee joints (Fig. 2*F*), the overall histopathological score (Fig. 2*E*), and serum levels of the proinflammatory cytokines IL-6, IFN-γ, and IL-17 (Fig. 2 *G*–*I*). Importantly, Dex also failed to reduce the swelling

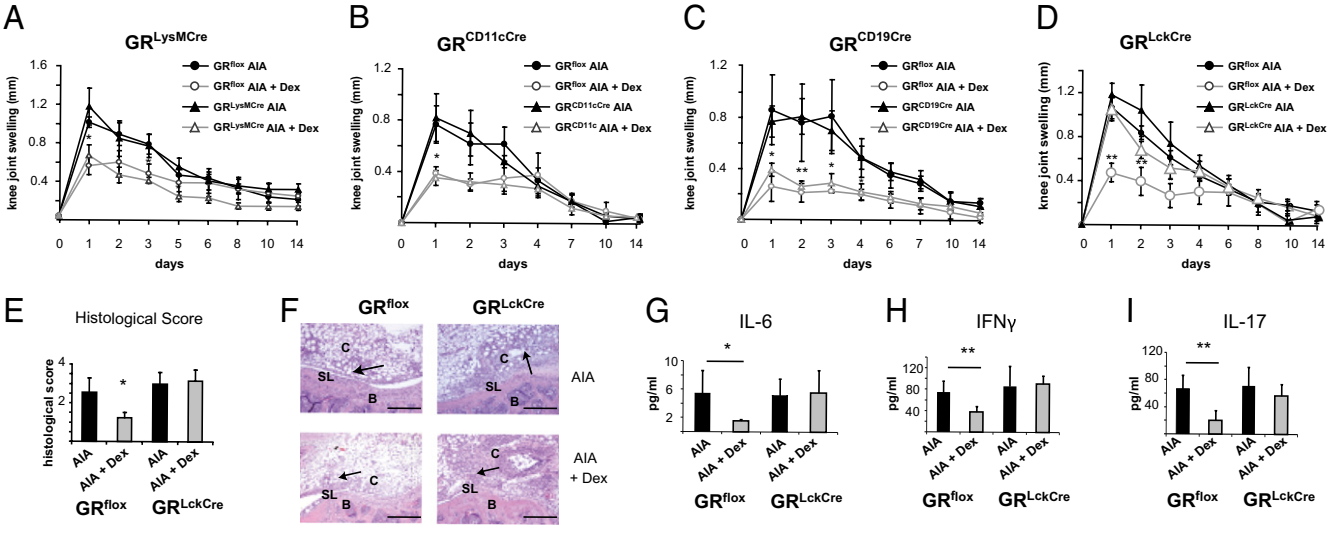

Fig. 2. GC-mediated suppression of AIA requires the GR in T cells. (*A*–*D*) Knee joint swelling of (*A*) $GR^{LysMCre}$, (*B*) $GR^{CD11cCre}$, (*C*) $GR^{CD19Cre}$, and (*D*) GR^{LckCre} mice and their respective littermate controls (GR^{flox}) subjected to AIA and PBS or Dex treatment. (*E*) Histological score of arthritic knee joints of PBS- and Dex-treated GR^{flox} and GR^{LckCre} mice at day 1. (*F*) Representative H&E stainings of arthritic knee joints of PBS- and Dex-treated GR^{flox} and GR^{LckCre} mice at day 1 (arrows indicate infiltrations of inflammatory cells) (Scale bars, 0.2 mm.) (*G*–*I*) Serum levels of (*G*) IL-6, (*H*) IFN-γ, and (*I*) IL-17 in PBS- and Dex-treated arthritic WT and GR^{LckCre} mice at day 1. In *A*–*E* and *G*–*I*, $n = 5$–6; $^{*}P < 0.05$, $^{**}P < 0.01$.

response and IL-17 level in GR^{LckCre} mice when applied after full establishment of the disease at day 1 (Fig. S4). Hence, these data demonstrate that T cells but not myeloid cells or B cells are the target cells for GC-mediated immune suppression of AIA.

Dimerized GR in T Cells Is Necessary for Antiinflammatory Effects. Using GR^{dim} mice with an impaired GR dimerization, we next addressed whether GR dimerization is critical for the antiinflammatory effects of GCs or whether transrepression by the GR is sufficient. Arthritic GR^{dim} mice exhibited a normal inflammatory response, but unlike their wild-type (WT) littermates, GC treatment completely failed to reduce knee joint swelling (Fig. 3*A*). Moreover, in comparison with controls, steroid therapy failed to reduce cellular infiltration and serum levels of IL-6, IFN-γ, and IL-17 in GR^{dim} mice (Fig. 3 *C–F* and Fig. S5). The histopathological score was not significantly reduced by Dex in both genotypes, albeit in WT mice the difference between Dex- and PBS-treated animals was close to significance (Fig. 3*B*) ($P = 0.07$). Thus, the dimerized GR is required for the antiinflammatory effects of GCs in AIA.

Next, we investigated whether GR dimerization is also required for GC action in other models of arthritis. GR^{dim} mice back-crossed to the DBA/1 background were subjected to glucose-6-phosphate isomerase-induced arthritis (G6PI-IA), a severe form of polyarthritis (21). Application of Dex starting at the onset of the disease (day 9) and continuing until day 15 efficiently suppressed the inflammatory score in WT mice, whereas it was only slightly reduced in GR^{dim} mice in the acute phase of G6PI-IA (Fig. S6). However, at later phases from day 15 onward, a reduction of the swelling response could also be observed in Dex-treated GR^{dim} mice, albeit at a lower efficiency (Fig. S6*A*). Taken together, these results demonstrate that the dimerization function of the GR is essential for suppressing acute inflammation in two arthritis models, indicating a general role of GR dimerization in steroid therapy of this disease.

Our analyses of GR^{dim} and GR^{LckCre} mice suggested that both GR dimerization and the presence of the GR in T cells are necessary for the antiinflammatory effects of GCs in arthritis therapy. To test whether the importance of GR dimerization is restricted to T cells, we crossed homozygous GR^{dim} mice (genotype $GR^{dim/dim}$) with GR^{LckCre} mice (genotype $GR^{flox/flox;LckCre}$). The resulting $GR^{dim/flox;LckCre}$ mice were heterozygous for the GR^{loxP} and the GR^{dim} allele and selectively expressed cre-recombinase in T cells. Thus, they carried a dimerization-deficient GR in T cells expressed from a hemizygous GR locus (Fig. S1*G*) but maintained a GR-loxP allele and consequently WT GR expression in all other cells. Both $GR^{dim/flox;LckCre}$ and $GR^{dim/flox}$ control mice developed severe arthritis, with a normal disease progression similar to that in WT mice (Fig. 3*G* compared with Fig. 3*A*). The induced knee joint swelling was efficiently suppressed by GCs in $GR^{dim/flox}$ mice only; Dex treatment of $GR^{dim/flox;LckCre}$ mice could not reduce the clinical signs (Fig. 3*G*). Thus, the dimerized GR in T cells is indispensable for the antiinflammatory effects of GCs in AIA therapy.

GCs Reduce T-Helper Cell Cytokines in Arthritic Mice but Do Not Induce Regulatory T Cells. We next investigated how GCs regulate T cells in AIA. GC treatment did not significantly affect total cell number (Fig. 4*A*) or $CD4^+$ cell number (Fig. 4*B*, *Left*) in the draining lymph nodes of WT and GR^{dim} mice. Accordingly, apoptosis and the proliferation rate of $CD4^+$ cells were unaltered under these conditions (Fig. S7). We then analyzed the capacity of T cells to respond to the antigen mBSA by flow cytometry of isolated draining lymph node cells from arthritic Dex-treated WT and GR^{dim} mice (day 1) restimulated ex vivo for 6 h with mBSA (Fig. S8*A*). Because CD154 (CD40 ligand) is up-regulated after T cell receptor triggering (22), the fraction of $CD154^+$ cells in response to mBSA treatment ex vivo represents the fraction of antigen-specific cells. The frequency of $CD154^+$ cells in Dex-treated WT mice showed a tendency to be reduced (Fig. S8*B*). Upon calculation of the total number of activated $CD154^+$ cells in the draining lymph nodes, a significant reduction was detected (Fig. 4*B*, *Right*). In contrast, there were no differences in the frequency and number of $CD154^+$ cells between Dex- and PBS-treated GR^{dim} mice.

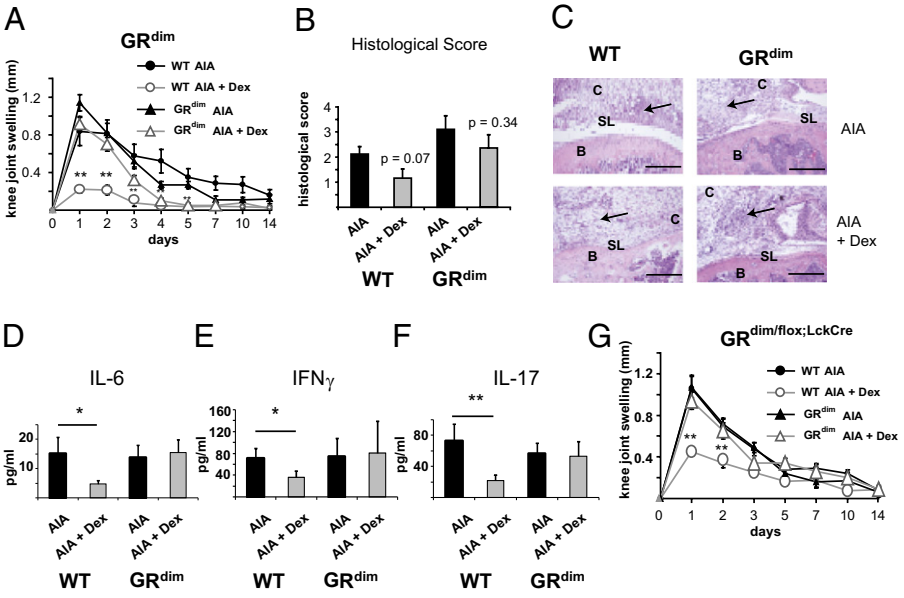

Fig. 3. GC-mediated suppression of AIA requires dimerization of the GR in T cells. (*A*) Clinical development of AIA in PBS- and Dex-treated WT mice and mice harboring a dimerization-deficient GR (GR^{dim}) determined from measurements of knee joint swelling. (*B*) Histological score of knee joints of PBS- and Dex-treated arthritic WT and GR^{dim} mice at day 1. (*C*) Representative H&E stainings of PBS- and Dex-treated arthritic knee joints of WT and GR^{dim} mice (arrows indicate infiltrations of inflammatory cells) (Scale bars, 0.2 mm.) (*D–F*) Serum levels of (*D*) IL-6, (*E*) IFN-γ, and (*F*) IL-17 measured in PBS- and Dex-treated arthritic WT and GR^{dim} mice at day 1. (*G*) Knee joint swelling of PBS- and Dex-treated arthritic $GR^{dim/flox}$ and $GR^{dim/flox;LckCre}$ mice, which lack the dimerized function of GR exclusively in T cells. In *A*, *B*, and *D–G*, $n = 5–7$; $*P < 0.05$, $**P < 0.01$.

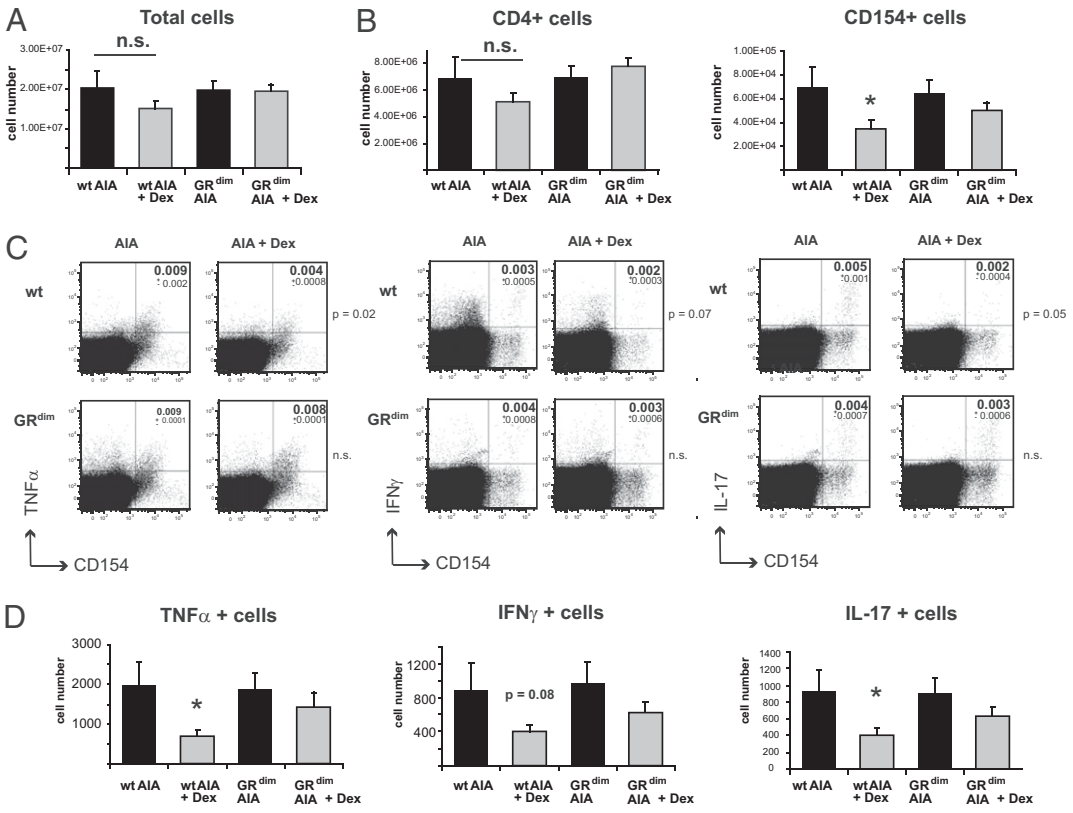

Fig. 4. GCs reduce T_H1 and T_H17 cell numbers in WT mice but not in GR^{dim} mice. (*A*) Total cell numbers of draining lymph nodes derived from arthritic PBS- or Dex-treated WT and GR^{dim} mice at day 1. (*B*) Cells were restimulated ex vivo for 6 h with mBSA, then stained with anti-CD4 and intracellularly with anti-CD154. The number of $CD4^+$ cells (*Left*) and $CD154^+$ cells (*Right*) was calculated using the frequency of $CD4^+$ cells and $CD154^+$ cells, respectively, from the total number of cells (shown in *A*). (*C*) Intracellular FACS staining of restimulated draining lymph node cells from *B*. Cells were stained with anti-TNFα (*Left*), anti–IFN-γ (*Center*), and anti–IL-17 (*Right*). Subsets of $CD4^+$ cells are represented, and *P* values are indicated to the right of plots. (*D*) Intracellular FACS analysis of mBSA-restimulated lymph node cells determining the numbers of cytokine-producing cells in the total number of cells. In all four panels, $n = 18$; $^*P < 0.05$; n.s., not significant.

As expected, a minor fraction of the $CD4^+$ cells produced TNF-α, IFN-γ, or IL-17 upon restimulation with mBSA (Fig. 4*C*). The percentage of TNF-α–producing $CD4^+$ cells was reduced in Dex-treated WT mice but not in GR^{dim} mice (Fig. 4*C*). IFN-γ was not significantly reduced in either genotype, although there was a tendency for stronger reduction in WT cells ($P = 0.08$). These results indicate that the antigen-induced T_H1 response is diminished in WT but not in GR^{dim} mice. T_H17 cells are known to play an important role in autoimmunity, particularly in RA (14, 15). The frequency of IL-17–producing $CD4^+$ cells was twofold lower in the draining lymph nodes of GC-treated WT animals. However, GC treatment failed to reduce the frequency of these cells in GR^{dim} mice (Fig. 4*C*). Although we did not observe any reduction of the percentage of TNF-α– and IL-17–producing cells within the fraction of $CD154^+$ cells (Fig. S8*C*), their absolute numbers were diminished in Dex-treated WT mice. Importantly, this reduction was absent in GR^{dim} mice (Fig. 4*D*). IFN-γ–producing cells were not significantly altered by Dex in both genotypes, albeit in WT mice the difference was close to significance (Fig. 4*D*).

Regulatory T cells are instrumental in preventing autoimmune diseases, including experimental AIA. Nonetheless, we could exclude an induction of regulatory T cells by GCs as a potential antiinflammatory mechanism, because the number of $CD4^+CD25^+FoxP3^+$ T cells was not increased in Dex-treated mice with AIA (Fig. S9 *A* and *B*). Thus, a reduction of T_H1 and T_H17 cells likely contributes to the antiinflammatory effects of GCs in AIA.

IL-17A$^{-/-}$ Mice but Not IFN-γ$^{-/-}$ Mice Are Resistant to GC Therapy. Because the numbers of T_H1 and T_H17 cells were reduced after GC treatment of AIA, we examined to what extent the suppression of T_H1 and T_H17 cytokines contributes to the antiinflammatory effects. We tested IL-17A$^{-/-}$ and IFN-γ$^{-/-}$ mice with AIA for their response to GCs. As expected (23), IFN-γ$^{-/-}$ mice developed an exacerbated disease, indicated by a significantly higher knee joint swelling compared with controls (Fig. 5*A*), caused by elevated IL-17 levels (Fig. 5*B*). Despite the 2.5-fold stronger knee swelling in IFN-γ$^{-/-}$ mice, Dex treatment was still able to significantly reduce the arthritis (Fig. 5*A*) and to suppress IL-17 levels (Fig. 5*B*). We then tested whether it is the reduction of IL-17 that is crucial for the antiinflammatory effects. IL-17A$^{-/-}$ mice showed a reduced knee joint swelling in comparison with controls at day 1, but not at subsequent days (Fig. 5*C*). Intriguingly, GC application did not further reduce the clinical signs of arthritis in IL-17A$^{-/-}$ mice, which were elevated compared with Dex-treated WT mice (Fig. 5*C*). Thus, mice deficient for IL-17 are resistant to Dex treatment.

Taken together, our findings demonstrate that GC therapy is dependent on the dimerized GR in T cells, which seems to be necessary to reduce the number of IL-17–producing cells.

Discussion

The present study shows that GR dimerization in T cells and a reduction of IL-17–producing cells are critical for the antiinflammatory effects of GCs in AIA, a mouse model exhibiting characteristic features of RA (17).

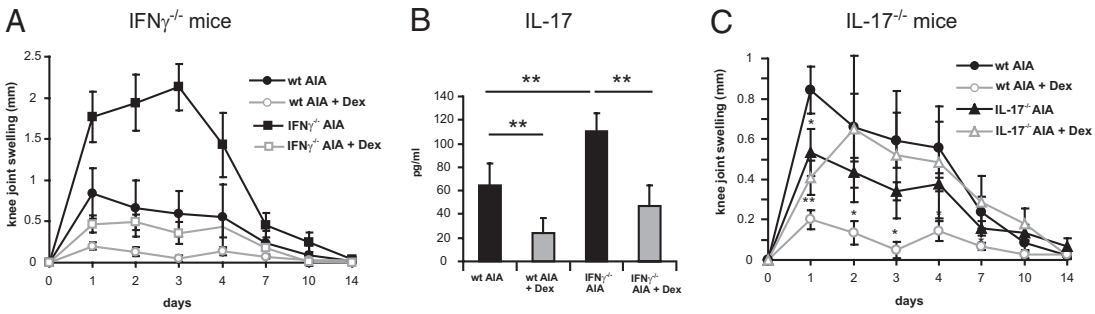

Fig. 5. GC response of IFN-$\gamma^{-/-}$ and IL-17A$^{-/-}$ mice. (*A*) Clinical development of AIA in PBS- and Dex-treated WT mice and mice deficient for IFN-γ determined from measurements of knee joint swelling. (*B*) Serum level of IL-17 measured in PBS- and Dex-treated arthritic WT and IFN-$\gamma^{-/-}$ mice at day 1. (*C*) Clinical development of AIA in PBS- and Dex-treated WT mice and mice deficient for IL-17 determined from measurements of knee joint swelling. In all three panels, $n = 5–7$; $^{**}P < 0.01$.

Although macrophages and neutrophils are considered key players in the pathology of human RA (24–26) and AIA (27, 28), we did not observe an impaired effectiveness of GC treatment in arthritic mice lacking the GR in myeloid cells or lacking the GR in B cells. The latter finding is in line with our observation that IgG titers in WT mice were not altered after Dex treatment in AIA and in the G6PI-IA model (Fig. S2). Although it has been reported that GCs reduce IgG titers in healthy people (29), inhibition of B cell activity and autoantigen production do not contribute to the treatment of AIA by GCs. GCs potently affect DC maturation, activation, and migration and cytokine release (4). However, in the context of a therapeutic application of GCs in AIA, the GR in DCs plays a minor role; mice deficient for the GR in CD11c^{+} DCs exhibited a potent suppression of AIA by GCs.

Our finding that GR$^{\mathrm{LckCre}}$ mice were completely resistant to GC-induced amelioration of AIA symptoms reveals that T cells are the most important targets of GCs in AIA. Interestingly, the partial recombination of the GR$^{\mathrm{loxP}}$ allele in T cells in GR$^{\mathrm{CD11cCre}}$ mice (Fig. S1 *A* and *B*) did not impair the therapeutic response to Dex. Only in GR$^{\mathrm{LckCre}}$ mice that exhibit an almost 100% recombination of the GR$^{\mathrm{loxP}}$ allele in T cells (Fig. S1*F*) we observed a complete resistance toward GCs.

Our study provides evidence that GR dimer-dependent processes, such as transcriptional transactivation, are involved in the suppression of T cell activation and that dimerization of the GR is essential for immune suppression in at least two arthritis models. GR$^{\mathrm{dim}}$ mice were completely resistant to GCs after induction of AIA and partially resistant after G6PI-IA induction. Although GCs were able to exert a partial antiinflammatory response despite the absence of GR dimerization in the later progression phase, GR dimerization proved to be critical in the acute phase of G6PI-IA. These findings suggest that dimerization of the GR in general might be required for the treatment of arthritis by GCs, challenging concepts that selective GR agonists that avoid GR dimerization maintain antiinflammatory efficacy in the treatment of RA. For example, the selective GR agonist compound A (CpdA), believed to transrepress NF-κB activity but not to transactivate GR dimer-dependent target genes, exhibits efficient therapeutic potential in collagen-induced arthritis (30), suggesting that GR monomers are sufficient for the suppression of inflammation in RA. However, there is evidence that CpdA does not fully circumvent the involvement of GR dimerization in the suppression of RA: it can repress NF-κB activity in a GR-independent manner via attenuation of IκBα degradation and MAPK activation in RA synovial fibroblasts (31), and it can induce expression of the antiinflammatory-acting phosphatase dual specificity phosphatase 1 (32). This strongly suggests that CpdA is not fully dissociative with regard to DNA dimerization. Thus, our findings further underscore that selective GR modulators need to be fully characterized regarding their cell type-specific functions before conclusions on their usefulness can be drawn.

The findings presented here demonstrate that impairment of GR dimerization restricted to T cells is sufficient to ameliorate the immune suppressive effects of GCs, indicating that GR dimer-dependent inhibition of T cell function is the underlying cause of this effect. Inhibition of proinflammatory transcription factors [e.g., AP-1 (33)] by the GR monomer (34) and even nongenomic effects of the GR at the T cell receptor (TCR) complex (35) have been hypothesized to be involved in the suppression of T cells. In particular, activated antigen-specific T cells expressing CD40L were reduced by Dex in AIA, suggesting a central role of this regulatory mechanism. Other mechanisms such as reducing T-cell numbers by apoptosis or impaired proliferation, as well as an induction of regulatory T cells, were not observed and therefore are unlikely to be involved. The lower number of activated CD40^{+} T cells is in line with the well-established observation that GCs suppress T cell activation in general in terms of IL-2 expression (36). A reduction of cytokine expression in T cells, however, does not seem to contribute to the efficacy of GC therapy: we did not detect diminished TNF-α, IFN-γ or IL-17 levels within the fraction of activated T cells. This is corroborated by our finding that IL-17 produced in in vitro-generated WT T_H17 cells was suppressed by only ≈20% after Dex treatment (Fig. S9 *C* and *D*).

Intriguingly, the frequency of antigen-specific T_H1 and T_H17 cells in draining lymph nodes was reduced in a GR dimerization-dependent manner. This is also true for TNF-α– and IL-17–producing cells that were reduced upon GC treatment in wild-type but not in GR$^{\mathrm{dim}}$ mice and reflected in part by our observation that GC treatment of AIA did not reduce serum levels of IL-6, IFN-γ, and IL-17 in GR$^{\mathrm{LckCre}}$ and GR$^{\mathrm{dim}}$ mice. However, the major cause of the antiinflammatory effects of GCs in AIA is repression of IL-17– rather than IFN-γ–producing cells. IFN-$\gamma^{-/-}$ mice developed a significantly more severe arthritis; however, Dex application was still able to ameliorate the disease to a degree comparable to that in WT mice. The enhanced severity of IFN-$\gamma^{-/-}$ mice is due to elevated IL-17 levels (23), which can be reduced by Dex, resulting in a milder arthritis. Of note, we cannot exclude that TNF-α is a critical target for immunosuppression by GCs, because we did not analyze TNF-α–deficient mice. In contrast, IL-17A$^{-/-}$ mice displayed a milder AIA on day 1 but still mounted a robust inflammatory response on the following days. AIA in IL-17A$^{-/-}$ mice was not altered by Dex treatment, whereas it strongly reduced the swelling response in WT mice. Of note, we cannot entirely exclude a "compensatory" inflammatory response in IL-17A$^{-/-}$ mice that differs from the type of response in WT mice. Nonetheless, the unresponsiveness of IL-17A$^{-/-}$ mice to Dex treatment and the reduction of IL-17 levels in WT and IFN-$\gamma^{-/-}$ mice, but not in GR mutant mice, strongly indicate that IL-17 is a major target for immunosuppression by GCs.

GCs are capable of suppressing T_H1 cytokines like IFN-γ and TNF-α by reducing STAT-4 activity through direct interaction, thereby inhibiting the T_H1 lineage-specific transcription factor T-bet (37, 38). To date, only a few studies have reported suppressive effects of GCs on T_H17 cells (39–41). The present study demonstrates this phenomenon in an arthritis model.

The identification of IL-17 as an essential target of steroid therapy in AIA suggests that interfering with T_H17 cells using more specific compounds (42, 43) could be sufficient to treat arthritis and avoid other steroid-associated side effects. Indeed, beneficial outcomes in RA patients treated with an IL-17–neutralizing antibody have recently been reported (44).

Materials and Methods

Mice. All animal experiments were performed in accordance with accepted standards of animal welfare and with permission of the responsible authorities of the Bundesland Thüringen in Germany. Origin and generation of used mouse strains is described in *SI Materials and Methods*.

AIA and G6PI-IA. AIA was effectuated as previously described (23) using 8- to 12-wk-old mice (BALB/c or C57BL/6 background). G6PI-IA was established as described elsewhere (21). Details for histological and serum analysis are described in *SI Materials and Methods*.

Lymph Node Cell Analysis. Cells from draining lymph nodes (inguinal and popliteal) were isolated 24 h after AIA induction and cultured with mBSA or 4α-phorbol 12-myristate 13-acetate and ionomycin, followed by brefeldin A treatment and subsequent analysis by flow cytometry. Details regarding proliferation and apoptosis determination are described in *SI Materials and Methods*.

T_H17 in Vitro Differentiation. Naïve $CD4^+TCR\beta^+CD62L^{high}CD44^{low}$ cells were purified from spleens and lymph nodes of BALB/c mice by flow cytometry sorting and cultured in the presence of anti-CD3, anti-CD28 anti–IL-4 (11B11), and anti–IFN-γ (XMG.1) antibodies, as well as IL-6, TGF-β, IL-1β, and TNF-α. After 7 d, cells were restimulated with plate-bound anti-CD3 and anti-CD28 antibodies for 6 h with or without Dex, in the presence of brefeldin A. Details are described in *SI Materials and Methods*.

Statistics. For all statistical analyses, a two-tailed Student *t* test was used. All data are presented as the mean ± SEM.

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