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Regulatory T cells control the transition from acute into chronic inflammation in glucose-6-phosphate isomerase-induced arthritis

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ABSTRACT

Objectives Glucose-6-phosphate isomerase (G6PI)-induced arthritis is a spontaneously remitting experimental arthritis model. It was hypothesised that regulatory T cells (Tregs) are involved in remission and their role in G6PI-induced arthritis was investigated.

Methods Tregs were depleted by injection of anti-CD25 before immunisation of DBA/1 mice with G6PI. The severity of arthritis was assessed clinically and histologically and the number and function of G6PI-specific T helper (Th) cells were determined by flow cytometry. Th cells and monocytes/macrophages were depleted using anti-CD4 or clodronate-containing liposomes.

Results Injection of anti-CD25 depleted Tregs transiently. Normal numbers of Tregs were restored 5 weeks after G6PI immunisation. Whereas arthritis started to resolve in control mice 3 weeks after immunisation with G6PI, severe arthritis was still present in the anti-CD25-treated mice 12 weeks after immunisation. The most striking ex vivo correlate of non-remitting arthritis was a strong increase in G6PI-specific Th cells 3 days after G6PI immunisation. This difference between treated and control mice declined at later time points. Depletion of CD4 cells ameliorated arthritis in controls but not in anti-CD25-treated mice. In contrast, clodronate-containing liposomes were an effective treatment in both groups.

Conclusions Tregs control the transition from acute self-limiting to non-remitting destructive G6PI-induced arthritis already in the preclinical disease stage. Once established, non-remitting destructive arthritis is not controlled by restoration of normal Treg numbers. These findings question the rationale of therapeutic approaches augmenting Treg number or function in established arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology. T lymphocytes are thought to trigger effector cells which mediate synovitis and the destruction of cartilage and bone. These effector cells, including B cells, monocyte/macrophages, fibroblasts, neutrophils and mast cells, initiate and maintain a network of cytokines and proinflammatory mediators. We have previously reported that immunisation of non-transgenic mice with the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (G6PI) induces severe peripheral symmetrical polyarthritis in normal mice with high incidence (>95%). In this model, the development of arthritis depends on T cells, B cells and innate immunity. CD4 T helper (Th) cells are crucial not only for the induction of the disease but also during the effector phase. Depletion of CD4 T cells in arthritic animals induces remission of arthritis. Arthritis develops quickly, starting at day 9 after immunisation. Clinical disease reaches a maximum around day 15 and then resolves slowly. Between days 30 and 40 after immunisation, overt signs of clinical arthritis completely disappear. Accordingly, histological severity of arthritis peaks at day 15. Infiltration by granulocytes, lymphocytes and plasma cells, fibrin exudation and cartilage and bone erosions are usually found in G6PI-induced arthritis at the peak of disease. The inflammatory activity resolves at later time points and regenerative processes and fibrosis start to occur. Thus, G6PI-induced arthritis is a self-limiting arthritis and the affected mice usually do not develop a chronic progressive polyarthritis and concomitantly debilitating joint destruction.

The aim of this study was to investigate the mechanism(s) leading to spontaneous resolution of G6PI-induced arthritis. Given their well-described protective role in different models of autoimmune diseases, we hypothesised that regulatory T cells (Tregs) are involved in this immunoregulatory process. Depletion of Tregs before immunisation with G6PI leads to a non-remitting course of G6PI-induced arthritis even after normal numbers of Tregs were re-established in the mice. Exacerbated and non-remitting arthritis was associated with an early burst of cytokine production by G6PI-specific Th cells. Our data indicate that Tregs are critical determinants of the transition from acute to chronic inflammation.

MATERIALS AND METHODS

Animals, induction of arthritis and treatment

DBA/1 mice were bred and maintained in our specific pathogen-free animal facility. All experiments were approved by the appropriate governmental authority and conducted in accordance with institutional and state guidelines. Recombinant human G6PI was prepared as previously described. Briefly, G6PI was expressed as a his-tagged protein in Escherichia coli BL21. After lysis of bacteria, the recombinant protein was purified from the lysate with nickel-nitrilotriacetic acid, extensively dialysed against phosphate-buffered saline (PBS) and filter sterilised. The purity and integrity of the protein

Additional data are published online only. To view these files please visit the journal online (http://ard.bmj.com) and find the article.

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was assessed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Mice aged 6–12 weeks were immunised subcutaneously at the base of the tail with 400 μg G6PI emulsified in 100 μl complete Freund’s adjuvant (CFA) (Sigma-Aldrich, Taukirchen, Germany). Animals were scored for clinical signs of arthritis (erythema, swelling, ankylosis) as indicated in the figure legends. For histopathological examination, whole mouse legs were fixed in 4% buffered formalin for 2 days and subsequently decalcified with EDTA for 14 days at 56°C. The legs were then paraffin-embedded and microsections of 1–2 μm were stained with haematoxylin and eosin according to standard procedures. Samples were viewed with a DMRB microscope (Leitz, Wetzlar, Germany) at ×25, ×50, ×100, ×200 and ×400 magnification by a pathologist who was blinded to the experimental set-up. The site and severity of the inflammation was noted and the latter was graded semiquantitatively in five steps from 0 (normal finding) to 4 (strong inflammation), as described previously.1 For this purpose, the infiltration of lymphocytes, plasma cells and macrophages, together with enlargement of the synovial lining, pannus formation and cartilage/bone destruction were taken into account. The infiltration of neutrophil granulocytes was regarded as a sign of more active inflammation and was therefore graded separately.

For depletion of Tregs, mice were injected with 400 μg anti-CD25 (PC61.5) 11 and 8 days before immunisation. Control mice were left untreated. The antibody was purified from hybridoma culture supernatant by passing it over a protein G column (GE Healthcare, München, Germany). The column was washed extensively with PBS and the bound IgG was eluted with 0.1 M glycine (pH 2.7). Eluted antibody was extensively dialysed against PBS and filter sterilised. The purity and integrity were checked using SDS-PAGE under reducing and non-reducing conditions. Modulation of arthritis using anti-CD4 or clodronate-containing liposomes is described in the online supplement.

**Anti-G6PI-Ig ELISA**

Titres of G6PI-specific antibodies were measured by ELISA as previously described.1 The titre was defined as the last dilution that gave an optical density which was higher than mean ±3SD than the background.

**Flow cytometry**

Tregs were analysed by staining with anti-CD4 (GK1.5), anti-CD25 (7D4) and anti-FoxP3 (FJK-16s) using FoxP3 staining buffer set (eBiosciences, Frankfurt, Germany). G6PI-specific T cell responses were assessed by intracellular staining of G6PI-stimulated lymph node cells. Details of the methods can be found in the online supplement.

**Statistics**

Data were analysed using the non-parametric Mann–Whitney U test with SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA) unless otherwise indicated. Graphs were generated using SigmaPlot 10.0 (Systat Software, Chicago, Illinois, USA).

**RESULTS**

**Transient Treg depletion by anti-CD25 treatment**

For depletion of Tregs, DBA/1 mice were injected with 400 μg anti-CD25 monoclonal antibody (mAb, clone PC61.5) 11 and 8 days before arthritis induction. Control mice were left untreated and both groups of mice were immunised with G6PI in CFA. At several time points after mAb treatment, cells from the draining lymph nodes were harvested, stained for CD4, CD25 and FoxP3 and analysed by flow cytometry to determine the extent and duration of Treg depletion (see figure 1A in online supplement). As shown in figure 1B in the online supplement, the frequency of FoxP3+ cells among CD4 Th cells was reduced to at least 50% compared with the controls until day 15. At day 25 the reduction was lower but still statistically significant, whereas at day 35 (ie, 43 days after the last mAb treatment) we detected only a minimal reduction of Tregs in depleted mice. Similar data were obtained from the analyses of spleens (data not shown). Interestingly, the treatment depleted both CD25+ and CD25- FoxP3+ Tregs, suggesting that the reduction of FoxP3+ cells was not solely due to antibody-dependent cytotoxicity but also to a block of interleukin 2 (IL-2) signalling which is mandatory for maintenance of peripheral Treg numbers.5 6 Thus, anti-CD25 treatment resulted in a transient and incomplete depletion of Tregs, as described for other models elsewhere.7–9

**Exacerbation and non-remitting disease course in anti-CD25-treated mice**

We next assessed the influence of Treg depletion on the clinical and histological severity of G6PI-induced arthritis. Clinical signs of arthritis were usually first detectable at day 9 after immunisation in both control and anti-CD25-treated mice, and arthritis severity peaked around day 15 in both groups of mice. In addition, there was no consistent difference in maximal arthritis severity. After that time point, the clinical course was dramatically different between the two groups. Whereas arthritis began to resolve slowly after approximately day 20 in the control mice, almost all of the anti-CD25-treated mice had maximal clinical arthritis scores for more than 6 weeks and still suffered from massive arthritis 82 days after the immunisation (figure 1A). Not only did clinical signs of inflammation such as redness and paw swelling persist in these mice, but they also developed joint deformations and ankylosis, which are normally not seen in G6PI-induced arthritis (figure 1B). These destructive changes were already observed by day 30, implying that they were not solely a consequence of the prolonged inflammation but rather represent a distinct phenotype of arthritis in Treg-depleted mice.

The large increase in the severity and duration of arthritis was confirmed histologically. There was a much denser infiltration of neutrophils, lymphocytes and macrophages resulting in a higher total histological score in Treg-depleted mice at day 30 after arthritis induction (figure 1C, left). At day 82, when infiltration with neutrophils or mononuclear cells was only rarely found in controls, there was still low-grade active inflammation, mainly consisting of mononuclear cells, in the joints of Treg-depleted mice (figure 1C, right). Despite the paucity of inflammatory cells, a strong pannus with densely packed activated fibroblasts was still present (figure 1D). This pannus tissue had the ability to cover and invade into the joint cartilage, even destroying the subchondral bone layer, similar to the pannus observed in severe cases of human RA. However, granulomas or fibrinoid necrosis were not observed and the enlargement of the synovial lining was mild to moderate.

Our data therefore show that induction of G6PI arthritis in Treg-depleted mice resulted in a switch from the usually acute self-limiting course to non-remitting destructive G6PI-induced arthritis. Arthritis persisted even at time points at which the Treg compartment was fully restored, suggesting that this chronic disease phenotype cannot be attributed simply to the absence of Tregs. What, then, causes chronic arthritis in Treg-depleted mice?5 6
anti-G6PI antibodies of different subclasses were detectable from day 9 on in both groups. G6PI-specific Ig levels reached a maximum at day 30 after immunisation in the control group and declined thereafter. In contrast, the titres of anti-G6PI IgG2a and IgG2b further increased in anti-CD25-treated mice and were maximal at day 81 after immunisation. These data show that transient depletion of Tregs leads to prolonged and persistent humoral autoimmunity.

**Increased humoral autoimmune response in anti-CD25-treated mice**

DBA/1 mice produce high titres of G6PI-specific Ig after immunisation, and we have previously shown that B cells and FcγR+ effector cells are required for the pathogenesis of G6PI-induced arthritis.1 2 We therefore measured levels of G6PI-specific IgG1, IgG2a and IgG2b antibodies at different time points after arthritis induction. As shown in figure 2 in the online supplement, anti-G6PI antibodies of different subclasses were detectable from day 9 on in both groups. G6PI-specific Ig levels reached a maximum at day 30 after immunisation in the control group and declined thereafter. In contrast, the titres of anti-G6PI IgG2a and IgG2b further increased in anti-CD25-treated mice and were maximal at day 81 after immunisation. These data show that transient depletion of Tregs leads to prolonged and persistent humoral autoimmunity.

**Figure 1** Exacerbation and chronicisation of glucose-6-phosphate isomerase (G6PI)-induced arthritis in anti-CD25-treated mice. DBA/1 mice were treated with anti-CD25 and immunised as described in the Materials and Methods section. (A) Animals were examined at the indicated time points for severity of clinical arthritis (redness, swelling). Each paw was scored on a scale of 0–2 (0, healthy; 1, erythema/swelling; 2, severe erythema/swelling), giving a maximal score of 8 for each mouse. Data shown are from one representative experiment (n=5 per group) out of three. (B) Front-paw (top) and hind-paw (bottom) of a control (left) or an anti-CD25 monoclonal antibody (mAb)-treated (right) mouse at day 82 after immunisation. (C) H&E-stained sections were scored for the infiltration of neutrophil granulocytes and features of inflammation, including pannus formation, in a semiquantitative manner. For each parameter a maximum score of 4 could be reached and a total histological score was calculated by adding both scores. The mean scores of all paws at day 30 (left) and day 82 (right) are shown. (D) Typical histopathological picture of joint destruction in an anti-CD25 mAb-treated mouse at day 82 after immunisation showing the intense pannus formation (long arrows, left panel) consisting mainly of activated fibroblasts (small arrows, right panel). Proliferation of small blood vessels (circles, right panel) and a slight enlargement of the synovial lining (between bold arrows, right panels) are also typical of synovitis. The pannus destroys the synovial cartilage completely and reaches the underlying bone (bold arrows, left). Focally, newly formed cartilage can be observed (oval, left panel). H&E staining; magnification: left ×50, right ×200. Data are presented as mean±standard error of mean. LN, lymph nodes.
Tregs control the early T cell response after immunisation with G6PI

In G6PI-induced arthritis, CD4 Th cells are obligatory for disease pathogenesis.1 We therefore assessed the number and function of G6PI-specific T cells in draining lymph nodes by flow cytometry. We used CD154 expression after a brief ex vivo stimulation with G6PI to identify G6PI-specific T cells. CD154 is upregulated within a few hours after T cell receptor triggering and expression of this molecule after brief restimulation is used to identify antigen-specific T cells, independently of their cytokine production.2

A small population (approximately 0.12% of CD4+ cells) expressed CD154 ex vivo after brief restimulation with G6PI 3 days after G6PI immunisation in control mice (figure 2A, upper left panel). A minority of these cells (approximately 12% of the CD4+ CD154+ cells) produced tumour necrosis factor α (TNFα) and very few, if any, Th cells produced other cytokines in response to G6PI at this early time point (figure 2A, upper panels). In anti-CD25-treated mice we found roughly fourfold higher frequencies of G6PI-specific T cells (approximately 0.5% of CD4 cells, figure 2A, lower left panel). G6PI-specific cells from anti-CD25-treated mice were not only increased in frequencies but also showed strikingly increased cytokine production: a higher proportion of these cells (approximately 26.5% of the CD4 CD154+ cells) produced TNFα, about 16% produced receptor activator of nuclear factor κB ligand (RANKL) and about 6% of the G6PI-specific Th cells produced IL-2 at this early time point after immunisation (figure 2A, lower panels). The increased absolute number of lymph node cells in Treg-depleted mice (data not shown), the higher frequency of CD154+ Th cells and the considerable increase in the frequency of cytokine producers among the CD154+ Th cells add up to a 60-fold higher number of RANKL+ Th cells, a 20-fold higher number of IL-2+ Th cells and a 10-fold higher number of TNFα+ Th cells (figure 2B) in CD25-depleted mice at day 3 after immunisation.

The G6PI-specific T cell pool later expanded in both groups, reaching a maximum at day 9 (see figure 2C and online figure 3 in CD25-depleted mice at day 3 after immunisation. Treg depletion did not foster the differentiation of a particular Th cell subset since the increased total number of cells producing IFNγ or IL-17 was solely the result of the increased number of G6PI-specific T cells.

A qualitative change in the cytokine production of these antigen-specific T cells was only observed at day 3 after immunisation (higher expression of TNFα, RANKL and IL-2) in Treg-depleted mice. Treg depletion did not foster the differentiation of a particular Th cell subset since the increased total number of cells producing IFNγ or IL-17 was solely the result of the increased number of G6PI-specific T cells.

The striking increase in the number and cytokine production of G6PI-specific T cells at day 3 after immunisation, however, was unexpected and prompted us to analyse the G6PI-specific T cells at even earlier time points.

Depletion of CD4 cells is ineffectual in the effector phase of G6PI-induced arthritis in Treg-depleted mice

To examine whether the aggravated Th cell response is the cause of the non-remitting destructive G6PI-induced arthritis in mice depleted of Tregs, we depleted CD4+ cells in vivo by administration of an anti-CD4 mAb. Treatment before immunisation completely prevented disease in both anti-CD25-treated and control mice (figure 3A). As in our earlier studies,1 CD4 depletion after the onset of clinical disease (days 11 and 14 after immunisation) was still effective in control mice (figure 3B). In stark contrast, the same treatment was completely ineffective in mice depleted of Tregs before immunisation (figure 3B). This suggests that the early burst of the pathogenic Th cell response in Treg-depleted mice activates a pathogenic effector cell population(s) which causes non-remitting destructive arthritis and is subsequently uninfluenced by CD4 depletion or the restoration of normal Treg numbers.

Given the enormous number of possibilities, it would have been beyond the scope of this investigation to identify exactly the drivers of non-remitting destructive arthritis in Treg-depleted mice. Considering the pathogenic role of the monocyte/macrophage/osteoclast lineage in arthritis, we chose to deplete this cell population in Treg-depleted and control mice. As shown in figure 3C, in both control and Treg-depleted mice, treatment with clodronate-containing liposomes after the onset of arthritis ameliorated the disease with approximately similar efficiency. Thus, monocytes/macrophages/osteoclasts do not only represent a common final path of arthritis in both groups of mice, but seem to become independent on constant triggering by Th cells in the Treg-depleted animals which develop non-remitting destructive arthritis.

DISCUSSION

CD4+ CD25+ FoxP3+ Tregs are crucial for the maintenance of peripheral tolerance. An inborn lack of Tregs results in severe autoimmune syndromes in both humans and mice. How Tregs prevent autoimmune disease and whether they can influence clinically manifest inflammatory diseases such as RA is unclear.
and highly controversial. The seemingly paradoxical finding that the number of Tregs is often increased in patients with autoimmune disease has been correlated with either compromised or enhanced Treg function in vitro. Resistance of effector cells against modulation by Tregs has been invoked as an explanation for the latter finding. Tregs can delay the onset and/or downregulate the severity of collagen-induced arthritis (CIA), antigen-induced arthritis and spontaneous arthritis in K/BxN mice. The role of Tregs in proteoglycan-induced arthritis is, however, less obvious. Our data differ significantly from all these findings and add an important novel aspect by showing that anti-CD25-mediated depletion of Tregs leads to a non-remitting destructive course of G6PI-induced arthritis. This is unique to G6PI-induced arthritis, which is normally acute and self-limiting with almost complete resolution of joint inflammation after 30–40 days. The importance of our results therefore

Figure 2  Increased glucose-6-phosphate isomerase (G6PI)-specific T cell responses in T regulatory cell-depleted mice early after immunisation with G6PI. After G6PI immunisation, cells from the draining lymph nodes (LN) were isolated at the indicated time points, restimulated with G6PI and analysed for expression of CD154, tumour necrosis factor α (TNFα), receptor activator of nuclear factor κB ligand (RANKL), interleukin 2 (IL-2), IL-17, interferon γ (IFN-γ) and granulocyte macrophage colony-stimulating factor (GM-CSF) by flow cytometry. (A) Expression of CD154 and cytokines in control (upper row) or anti-CD25-treated (lower row) mice at day 3 after immunisation. (B) Fold change of expression of CD154, TNFα, RANKL and IL-2 in anti-CD25-treated mice compared with control mice at the indicated time points after immunisation. (C) Absolute numbers of total G6PI-specific (CD154+) cells and G6PI-specific cells expressing the indicated cytokines in control (black bars) or anti-CD25-treated mice (grey bars). At each time point a minimum of three mice per group was analysed. Data are representative of at least two independent experiments for each time point. Flow cytometry plots in (A) show concatenated data files from three mice per group.
lies in the demonstration that Tregs regulate the transition from acute to chronic inflammation. Treg depletion in G6PI-induced arthritis will therefore be used in further studies to examine the cellular and molecular determinants of chronicity in arthritis.

We have analysed T and B cell responses in Treg-depleted and control mice over the time course of arthritis to determine possible pathways leading to chronic G6PI-induced arthritis. The major findings from these analyses were (1) an enhanced and prolonged humoral anti-G6PI response; (2) a slightly increased number of G6PI-specific cytokine-producing Th cells in the effector phase; and (3) a strikingly increased number of G6PI-specific T cells in the early induction phase of the disease. Each of these results could contribute to the chronicity of arthritis.

We and others have shown previously that B lymphocytes and antibodies are necessary but not sufficient for the pathogenesis of G6PI-induced arthritis. Even the more severe arthritis in K/BxN mice deficient in FoxP3 is not mediated by an increased level or enhanced pathogenicity of the disease-inducing G6PI-specific IgG. Together with the fact that blockade of T cell costimulation via inducible costimulator (CD278) or CD28 can profoundly ameliorate arthritis without affecting anti-G6PI Ig levels. Taken together, it seems unlikely that the higher titres of anti-G6PI were the sole explanation for the chronicity of the disease.

The Th cell response was strikingly different at day 3 after immunisation between CD25-depleted and control mice. Importantly, this difference was present only at this very early time point. At later time points such as day 9, when T cell responses are typically assayed, the magnitude and quality of the Th cell response was very similar in both groups of mice. The high number of cytokine-producing G6PI-specific T cells 3 days after immunisation in Treg-depleted mice was a surprising finding which caused us to examine the presence of G6PI-specific Th cells even earlier—that is, in Treg-depleted mice that were not experimentally immunised with G6PI. The number of Th cells specific for a certain peptide/MHC complex has been estimated to vary between 20 and 200 in the secondary lymphoid organs of a mouse (0.1–1 cells in 10^6 total cells). Even if one considers that G6PI contains several epitopes that are recognised by CD4+ Th cells in DBA/1 mice, their number can be estimated to be <10 cells in 10^6 total cells, which is below the detection limit of our assay. Indeed, we never detected a number of CD154+ cells which was significant above background staining in non-immunised control mice. Thus, the detection of about 5500 G6PI-specific Th cells in the lymph nodes of Treg-depleted mice alone is not sufficient for the induction of autoimmune disease models but seems to facilitate it. The data presented here, however, go significantly beyond that by demonstrating the expansion and activation of antigen-specific effector Th cells in non-immunised Treg-depleted mice as a correlate of this increased disease susceptibility. Together with the markedly enhanced Th cell response in CD25-depleted mice at day 3 after immunisation, this suggests that Treg depletion allows for an early burst of pathogenic Th cell activation which sets the stage for non-remitting destructive arthritis. This inflammation
persists even after the subsequent restoration of normal Treg numbers in CD25-depleted mice (about day 35 after immunisation). Moreover, the non-remitting destructive arthritis in the CD25-depleted mice was not influenced by depletion of CD4 cells during the effector phase. This implies that the effector phase of G6PI-induced arthritis in Treg-depleted mice is much less T cell-dependent than in control mice. This is mirrored by the distinct clinical phenotype of disease in anti-CD25-treated mice with development of joint deformities and ankylosis which do not usually occur in G6PI-induced arthritis. Taken together, the pathogenesis appears to differ remarkably between the two cohorts of mice. This is further supported by the fact that depletion of monocytes, macrophages or osteoclasts was therapeutically effective at a time when CD4 depletion had no effect on the chronic arthritis in Treg-depleted mice. Considering the broadly depleting effects of clodronate-containing liposomes and the remarkable bone destruction in Treg-depleted mice, it is tempting to suggest that a significant part of the effect of Treg depletion on G6PI-induced arthritis is mediated via increased numbers of osteoclast precursors or numbers. Tregs suppress osteoclast formation in vitro, and it has been shown that suppression of CIA by adoptively transferred Tregs is paralleled by reduced osteoclast precursors but not by a reduced anticolagen antibody response. Osteoclastogenesis is known to be triggered by activated T cells (reviewed by Udagawa), mainly via their expression of RANKL. The striking increase in the number of G6PI-specific Th cells co-expressing RANKL and TNFα found in the early induction phase of G6PI-induced arthritis in Treg-depleted mice could recruit, expand or differentiate osteoclast precursors which, in turn, trigger the chronic destructive joint inflammation in these mice. Indeed, we found increased numbers of CD11b+ myeloid cells—which mainly contain osteoclast precursors—in the spleens of Treg-depleted mice (data not shown).

There are several implications of these findings for human RA. We have shown that the chronicity of arthritis is preceded by a very early burst of activity of the pathogenic autoimmune Th cells in G6PI-induced arthritis. That biomarkers of inflammation precede the development of RA by years is consistent with the idea that similarly critically dysregulated T cell responses occur in RA years before clinical disease. In other words, our data in the G6PI-induced arthritis model strongly suggest that the major impact of Tregs is in the early or even preclinical disease stages of arthritis. This hypothesis is supported by the fact that non-remitting destructive arthritis develops even in the presence of normal Treg numbers, as presented here, and the relatively small effect of enhancing Treg function in established arthritis in non-lymphopenic mice in vivo. If this was also the case in humans, the key to regaining control over inflammation in diseases such as RA would not be to add Tregs back (as is currently aimed at in a number of clinical and preclinical studies), but rather to identify and subsequently modulate those elements of the pathogenic immune response which dominate when Treg control has failed.

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