Published in final edited form as:

Immunol Lett. 2008 September 30; 120(1-2): 1–5. doi:10.1016/j.imlet.2008.07.008.

Regulation of autoimmune inflammation by pro-inflammatory cytokines

Eugene Y. Kim¹ and Kamal D. Moudgil^{1,2}

¹Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

The pro-inflammatory cytokines play a critical role in the initiation and propagation of autoimmune arthritis and many other disorders resulting from a dysregulated self-directed immune response. These cytokines influence the interplay among the cellular, immunological and biochemical mediators of inflammation at multiple levels. Regulation of the pro-inflammatory activity of these cytokines is generally perceived to be mediated by the anti-inflammatory and immunosuppressive cytokines such as IL-4, IL-10, or TGF- β . However, increasing evidence is accumulating in support of the regulatory attributes of the pro-inflammatory cytokines themselves, in studies conducted in animal models of diabetes, multiple sclerosis, uveitis, and lupus. The results of our recent studies have shown that the pro-inflammatory cytokines, TNF- α and IFN- γ , can suppress arthritic inflammation in rats, and also contribute to resistance against arthritis. These results are of paramount significance not only in fully understanding the pathogenesis of autoimmune arthritis, but also in anticipating the full ramifications of the in vivo neutralization of the pro-inflammatory cytokines, including that for therapeutic purposes.

Autoimmune diseases such as multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), and rheumatoid arthritis (RA) represent prototypic T cell-mediated diseases. In these and other T helper 1 (Th1)-mediated disorders, the pro-inflammatory cytokines are typically considered to be pathogenic, whereas the anti-inflammatory cytokines are regarded to be protective in nature [1-3]. However, this functional grouping of cytokines might be an oversimplification. Many cytokines are now known to have pleiotropic and/or redundant effects as well as counter-intuitive, opposing effects even for the same cytokine [4-9]. In this review, we highlight the paradoxical anti-inflammatory effects of the prototypic pro-inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interferon- γ (IFN- γ), in autoimmune inflammation. Most of our discussion is based on experimental models of arthritis, with additional relevant examples from other autoimmune model systems.

The cytokines of relevance in the pathogenesis of autoimmune arthritis in humans and rodents

RA is a multifactorial, chronic autoimmune disease of humans, and it is characterized by symmetrical involvement of joints. The infiltration of mononuclear cells into the synovium

²Corresponding author: Kamal D. Moudgil, Department of Microbiology and Immunology, University of Maryland School of Medicine, Howard Hall Room 323 C, 660 West Redwood St., Baltimore, MD 21201; Fax: 410-706-2129; E-mail: kmoud001@umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and the degradation of the cartilage and bone are caused by a variety of inflammatory mediators, including cytokines [10,11]. Two of the pro-inflammatory cytokines that play a critical role in RA are TNF- α and interleukin-1 β (IL-1), which up-regulate each other in a positive feedback loop to cause most of the pathologic symptoms. These cytokines are the major inducers of proliferation of pannus-resident fibroblasts that produce collagenase and other proteolytic enzymes, which in turn cause cartilage degradation. Furthermore, these cytokines are activators of osteoclasts for bone demineralization and also promote angiogenesis. In addition, these cytokines can cause systemic symptoms such as malaise, fatigue, and elevation of acute phase reactants in serum. Anti-cytokine therapies such as anti-TNF- α agents, including Etanercept, TNF receptor/immunoglobulin fusion protein and Infliximab (an anti-TNF- α antibody) [1, 12] have been successful in the treatment of RA. However, Kineret, a recombinant IL-1 receptor antagonist (IL-Ra) that competitively binds to the IL-1 receptor [13,14], has not yet yielded the optimal and expected benefits using the available test products. Other cytokines of relevance in RA include IFN- γ and granulocyte-macrophage colony stimulating factor (GM-CSF), both of which can activate macrophages [10,11]. On the other hand, the downregulation of inflammation can occur by the production of IL-10 and TGF- β , but the *in vivo* role of these cytokines in RA is not fully clear [10,11].

In the recent years, IL-23/IL-17 axis has been shown to play a major role in the pathogenesis of autoimmunity such as experimental autoimmune encephalomyelitis (EAE) [7,15,16], type 1 diabetes mellitus (T1D) [17,18], and uveitis [19]. In RA, IL-17 was detected in the synovium and this cytokine has been shown to synergize with IL-1 to induce IL-6 production by synovial fibroblasts, as well as enhance the expression of specific chemokines in the joint tissue [20, 21]. IL-17 is produced by T cells (T helper 17; T_h17) that belong to a different lineage than T_h1 and T_h2 cells, and it can also stimulate the secretion of TNF- α , IL-1 β , and chemokines in macrophages and other cell types [22,23]. Transforming growth factor- β (TGF- β) and IL-6 [24,25] induce the differentiation of T_h17 cells, whereas IL-23 secreted by antigen-presenting cells (APCs) facilitates the expansion and maintenance of this subset of T cells. In experimental arthritis models, IL-17 was shown to be an important mediator of inflammation and joint destruction in both collagen-induced arthritis (CIA) [26] and a model of spontaneous arthritis in IL-1Ra deficient mice [27]. Furthermore, a study in the AA model showed that IL-17 was expressed transiently in the lymph nodes during early part of the disease [28].

The temporal kinetics of the cytokine responses of arthritic Lewis (LEW) rats during the course of adjuvant arthritis (AA)

AA is an animal model for human RA, and it shares many of the clinical and histopathological features with the human disease. AA is inducible in Lewis (LEW) (RT.1^l) rats by s.c. injection of heat-killed *M. tuberculosis* H37Ra (Mtb) [29], and arthritic rats develop T cell response to mycobacterial heat-shock protein hsp65 (Bhsp65). Furthermore, the T cells directed against the determinant region 180-188 (B180)/177-191 (B177) of Bhsp65 represent the pathogenic subset of T cells.

In AA, the draining lymph nodes are the major site of priming of T cells following Mtb injection. The pathogenic T cells are activated there during the incubation phase of AA. The lymph nodes might also be the site where the disease-regulating T cells are primed during the course of AA. In addition, besides the T cells, macrophages and dendritic cells (DC) also play an important role in the pathogenesis of arthritis. Therefore, study of the cytokine profile of the draining lymph node cells (LNC) containing T cells, macrophages and DC is critical for obtaining insights into the naturally occurring cytokine milieu *in vivo*. Only a couple of studies have examined the temporal profiles of cytokines directly from serum, lymph nodes, and/or joints during the course of AA [28,30].

A study on the cytokines present within the joint homogenate and the serum of LEW rats showed that TNF- α and IL-1 β appeared in the serum at the onset of AA [30], whereas the same cytokines were detectable in the joints closer to the peak of the disease. In comparison, IL-6 appeared in both the serum and the joints during the recovery phase of AA [30]. Another study [28] based on the testing of various cytokines expressed in the inguinal lymph nodes during different stages of AA revealed that TNF- α and IL-17 were expressed at significant levels only before the onset of AA. However, IFN- γ revealed a biphasic profile such that it was significantly upregulated before onset of the disease with subsequent slight decrease, followed again by higher expression levels through the recovery phase. On the other hand, no changes in the level of IL-4 or TGF- β were noted [28]. However, in the joints, the cytokine levels differed significantly, with TNF- α and TGF- β levels peaking at the onset of AA, while IFN- γ just appearing only at the onset of arthritis. In contrast, IL-4 appeared around peak of AA, but there was no change in IL-17 expression.

Profiles of cytokine secretion in response to Bhsp65 in AA-susceptible LEW versus AA-resistant Wistar Kyoto (WKY) rats

Interestingly, the WKY (RT.1^l) rats having the same MHC haplotype as the LEW rats are resistant to AA despite their ability to raise a potent immune response to Bhsp65 following Mtb injection. The immunologic basis of regulation of acute AA in LEW rats and of resistance to AA of WKY rats has not yet been fully defined. Furthermore, there is meager information on the antigen-specific cytokine profiles of the draining LNC at different phases of AA, or on the comparative cytokine profiles of AA-susceptible and AA-resistant rat strains.

We examined this issue using Mtb-immunized LEW and WKY rats [31,32]. AA is considered to be a typical T_H1-mediated disease, in which IFN- γ and TNF- α play major roles in disease induction/propagation. Accordingly, we anticipated a predominantly T_H2 (IL-4 and IL-10) or T_H3 (TGF- β) type of response to mediate both the recovery from AA in the LEW rats and the resistance against AA in the WKY rats. Surprisingly, the cytokines secreted by LEW rats in the recovery phase and by WKY rats early after Mtb challenge were predominantly of T_H1 type [31,32]. Thus, enhanced secretion of Th1 cytokines positively correlated with regression of acute inflammation in LEW rats and protection (resistance) against arthritis in WKY rats. These findings were entirely unexpected given the Th1-dominant milieu in AA.

Experimental downmodulation of arthritis and other autoimmune diseases by pro-inflammatory cytokines

In our recent studies based on the AA model [31,32], we observed that both IFN- γ and TNF- α can significantly down-modulate the course of arthritis. The examinations of the mechanisms by which these cytokines can ameliorate arthritis have shown distinct regulatory pathways leading to a similar final outcome: disease modulation. Peptide-induced IFN- γ suppressed IL-17 production by the pathogenic T cells [31,32], whereas TNF- α treatment significantly reduced the production of IFN- γ by the pathogenic T cells (Figure 1).

a) IFN- γ

To test the effects of IFN- γ in LEW rats, we employed an AA-modulatory peptide of Rhsp65 (R465) that induces a predominantly Th1-secreting T cells [33] instead of using the toxic purified cytokine itself, to mimic a future clinical situation of peptide immunotherapy in the clinic. We observed an increase in IFN- γ after B177 recall [31]. However, this higher T_H1 response to B177 was accompanied by a decrease in IL-17 produced by B177-reactive T cells [31]. Our results are supported by those of other investigators showing that the administration of recombinant IFN- γ leads to reduced severity of AA and improved recovery from the disease

[34]. Similarly, the injection of anti-IFN- γ antibodies either to naïve rats before the induction of AA [35] or to recipient LEW rats prior to the adoptive transfer of arthritogenic LNC [36] aggravated the severity of arthritis. In addition, antigen-induced T cells that are capable of suppressing AA have been shown to secrete IFN- γ and IL-10 [37,38]. Furthermore, IFN- γ KO [39] and IFN- γ receptor KO [40,41] mice exhibited increased severity of CIA. Interestingly, CIA-resistant B6 strain can be rendered susceptible to arthritis by knocking out IFN- γ , and this sensitivity is also associated with higher IL-17 production [42]. Also, in EAE, IFN- γ receptor KO mice failed to recover from the disease [43].

Studies in other models of autoimmunity have also provided evidence for the immunoregulatory role of IFN- γ and IL-12 tested by direct injection of these cytokines into mice or by comparing the disease severity in mice lacking IFN- γ or IFN- γ receptor (IFN γ R). The injection of IFN- γ along with TNF- α into NOD/Wehi mice led to an upregulation of MHC I and II on the ductal and acinar cells of the pancreas as well as a marked reduction in the severity of insulinitis [44]. Recently, it has been shown that IFN- γ induced following immunization with a construct of immunoglobulin and a peptide of glutamic acid decarboxylase (GAD) suppressed insulinitis and restored blood glucose levels by suppression of pathogenic IL-17 [45]. In a study in experimental autoimmune uveitis (EAU), IL-12 administration was found to suppress the disease in susceptible mouse strains [46]. The timing of IL-12 injection was critical in that treatment during the first week of a uveitogenic antigenic challenge was much more effective compared to that in the second week, showing the significance of in vivo temporal events related to disease pathogenesis. The protective effect of IL-12 in EAU was shown to involve enhanced production of IFN- γ , nitric oxide, and apoptosis of uveitogenic effector T cells. Moreover, this protection was compromised in IFN- γ -deficient mice. IFN- γ has also been reported to be protective against experimental autoimmune encephalomyelitis (EAE). BALB/c mice lacking IFN- γ and C57BL/6 mice deficient in IFN γ R were rendered susceptible to myelin-basic protein (MBP)-induced EAE, whereas their wild type counterparts failed to develop the disease [47]. Furthermore, IFN- γ was shown to modulate the expression of certain key chemokines relevant for EAE pathogenesis. Similarly, intrathecal injection of IFN- γ was associated with reduced severity of EAE as well as enhanced recovery from the disease, and the extent of the protective effect observed was dependent on the timing of cytokine injection [48]. Using a model of chronic viral myocarditis, it was shown that the severity of the disease was markedly enhanced in mice lacking IFN- γ than in wild type mice [49].

The above studies coupled with ours [31,32] show that IFN- γ can regulate autoimmune inflammation. One mechanism of this protective effect is via regulation of IL-17. In addition, IFN- γ may mediate its effects via the upregulation of suppressor of cytokine signaling 1 (SOCS1, a regulatory protein that attenuates IFN- γ signaling) [50], the inhibition of proliferation of T cells and macrophages [43,51], and the induction of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) [43,52]. NO has been shown to inhibit the proliferation of T cells by preventing the activation of Janus kinases [53], and to promote the apoptosis of inflammatory cells [54]. Furthermore, T_H1 effector cells are more likely to undergo apoptosis than T_H2 cells [55], thereby increasing the probability of IFN- γ induced NO-mediated death of pathogenic effector T cells [46]. Both IFN- γ and TNF- α have also been shown to induce apoptosis of diabetogenic T cells in BCG-induced protection against diabetes [56]. In addition, IFN- γ , especially in conjunction with TNF- α , upregulates the expression in dendritic cells of IDO, which catabolizes tryptophan and prevents the proliferation of adjacent T cells, and thus can suppress the development of pathogenic T cells [57,58]. IFN- γ has also been proposed to regulate the production of pro-inflammatory mediators like IL-1 β [39] and prostaglandin E2 (PGE2) [59]. Furthermore, IDO has also been invoked in the generation of regulatory T cells [57,58], linking the modulatory interplay between IFN- γ , IDO, and Treg.

b) TNF- α

Injection of TNF- α into LEW rats led to the downmodulation of AA [32]. This inhibition of AA was not a result of general immunosuppression by high concentration of TNF- α administered, an increased release of soluble TNF-receptor-I (sTNFR) in the serum, or the generation of anti-TNF- α antibodies following TNF- α injection. In addition, there was no evidence for an altered migration of T cells into the peritoneum (the site of TNF- α injection), an increase in the indoleamine 2,3-dioxygenase (IDO) expression in splenic APCs, or an increase in the number of CD4+CD25+ regulatory T cells (Treg) within the peripheral blood (unlike the results of another study [60]). Nevertheless, there was a decrease in IFN- γ secretion by B177-specific T cells. This decrease in IFN- γ secretion could occur in part via TNF- α -mediated negative regulation of IL-12 production [13]. We suggest that TNF- α may also mediate its effects by influencing both - i) the apoptosis of pathogenic T cells that mediate arthritis induction [61], and ii) the migration of inflammatory cells into the joints by changing the expression of adhesion molecules on endothelial cells [62]. These aspects of TNF- α as well as the influence of pro-inflammatory cytokines in modulation of the activity of Treg [60,63] in the AA model remain to be tested.

The pivotal role of TNF- α in human RA is evident by the success of anti-TNF- α -based therapeutic approaches [1,12]. However, several pieces of evidence from studies in animal models of arthritis point to the anti-inflammatory or immunoregulatory role of TNF- α in arthritis. For example, it has been shown that mice lacking TNF- α [64] and those deficient in TNF-receptor 1 (TNFR1) [65,66] can develop CIA, although with reduced frequency compared to wild type mice. In TNFR1-deficient mice, the progression of arthritis in the affected joints was similar to that in wild type mice, suggesting that only certain features of the disease were critically dependent on TNF- α [65]. Additional studies using TNF- α neutralization using TNFR1-IgG1 fusion protein at different stages of CIA revealed that the dependence and effects of TNF- α in arthritis were stage dependent [65]. TNF- α injection of TNFR1-deficient mice in early initiation phase of CIA aggravated the disease, whereas TNF- α administration in late initiation phase suppressed arthritis, again pointing to the opposite stage-dependent effects of this cytokine [66]. A study employing in vivo neutralization of TNF- α in the AA model also revealed differential effects on arthritis of the dosage as well as the timing of administration of sTNFR [67]. Taken together, these studies highlight the significance of the effects of temporal variations in TNF- α production during the course of arthritis and the resulting effects of the in vivo blockade of the cytokine. Superimposed on this variable may be the inter-individual differences in the hypothalamic-pituitary-adrenal (HPA) axis that can modulate its interplay with TNF- α [68]. These factors could contribute to the differential benefits of TNF- α blockade therapy in a heterogeneous human population with subsets of RA patients showing much higher benefit than others. Furthermore, there is some evidence for anti-inflammatory effect of TNF- α in regard to infections, for example, listeria [69] and malaria [70]. This observation might help explain the side effect in a subset of patients of increased susceptibility to certain infections associated with anti-TNF- α therapy. However, despite a convincing evidence for the regulatory roles of TNF- α in animal models of arthritis, diabetes and EAE, it is rather difficult to fully rationalize the contrasting side of the success of anti-TNF- α therapy. We believe that the answers to this important question would come from further studies in subsets of RA arthritis patients that benefit from anti-TNF- α therapy compared to those that do not.

Immune regulation by TNF- α has been observed in other models of autoimmune diseases as well. Using a mouse model of systemic lupus erythematosus (SLE), it was shown that the severity of the disease was genetically linked to the reduced levels of TNF- α , and the replenishment by recombinant TNF- α significantly delayed the development of nephritis [71]. Studies in the non-obese diabetic (NOD) mouse model of type 1 diabetes mellitus (T1D)

have unraveled the protective effects of TNF- α . Injection of recombinant TNF- α was shown to suppress insulinitis as well as the development of diabetes [72,73], and the TNF- α treatment could afford protection not only against spontaneously developing diabetes, but also against adoptively transferred diabetes [73]. In another set of studies, TNF- α expression within the pancreas prevented T1D in the NOD mice by abrogating the development of islet-specific pathogenic effector T cells [5,74]. Interestingly, the timing of the induced expression of TNF- α had a marked effect on the outcome such that the early expression aggravated diabetes, whereas the late expression afforded protection against the disease [5]. These results not only highlight the differential sensitivity to TNF- α of the temporal pathogenic events during the course of autoimmune diabetes, but also have implications in designing immunotherapeutic strategies aimed at altering the in vivo TNF- α levels in various autoimmune disorders. Similarly, protection against diabetes in NOD mice by bacillus Calmette Guerin (BCG) injection has been attributed to both IFN- γ and TNF- α [56]. In the EAE model, TNF- α knockout (KO) mice developed more severe myelin oligodendrocyte glycoprotein (MOG)-induced EAE, while TNF- α treatment significantly reduced the clinical severity of the disease [75].

Concluding remarks

Our results show that high levels of IFN- γ and TNF- α have a regulatory role in AA [31,32]. However, it is important to note that the IFN- γ -mediated suppression of IL-17 as well as the TNF- α -mediated suppression of IFN- γ secretion by B177-specific T cells was concluded from results of studies in which the modulation of arthritis was a result of a preventive rather than a therapeutic regimen. Furthermore, these cytokines are well documented to play a role in the induction and maintenance of inflammation [1-3]. These contrasting roles of pro-inflammatory cytokines can be reconciled by a model (Fig. 1) in which a particular threshold of each of these cytokines is required for the initiation of inflammation, but the secretion of these cytokines beyond their respective critical levels triggers regulatory mechanisms that suppress the ongoing inflammation [31,32]. In addition, we suggest that these regulatory mechanisms are compartmentalized so as to differentially regulate various T cell responses ranging from naïve T cell activation to memory responses. Taken together, our results provide novel insights into the immunoregulation of autoimmune arthritis by the pro-inflammatory cytokines, as well as provide a framework to anticipate and understand the ramifications of in vivo neutralization of pro-inflammatory cytokines for therapeutic purposes.

Acknowledgements

This work was supported by grants from the National Institutes of Health (NIH), Bethesda, MD and the Arthritis Foundation (National Office, Atlanta, GA and Maryland Chapter, Baltimore, MD). We thank Amitabh Gaur, Howard Chi, and Shailesh Satpute for helpful discussions.

References

1. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol* 2001;19:163–96. [PubMed: 11244034]
2. O'Shea JJ, Ma A, Lipsky P. Cytokines and autoimmunity. *Nat Rev Immunol* 2002;2:37–45. [PubMed: 11905836]
3. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003;3:133–46. [PubMed: 12563297]
4. Trembleau S, Penna G, Gregori S, Giarratana N, Adorini L. IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-gamma-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12-induced IFN-gamma. *J Immunol* 2003;170:5491–501. [PubMed: 12759426]

5. Christen U, Wolfe T, Mohrle U, Hughes AC, Rodrigo E, Green EA, et al. A dual role for TNF- α in type 1 diabetes: islet-specific expression abrogates the ongoing autoimmune process when induced late but not early during pathogenesis. *J Immunol* 2001;166:7023–32. [PubMed: 11390446]
6. Gor DO, Rose NR, Greenspan NS. TH1-TH2: a procrustean paradigm. *Nat Immunol* 2003;4:503–5. [PubMed: 12774069]
7. Steinman L. A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007;13:139–45. [PubMed: 17290272]
8. McDevitt H, Munson S, Ettinger R, Wu A. Multiple roles for tumor necrosis factor- α and lymphotoxin α/β in immunity and autoimmunity. *Arthritis Res* 2002;4:S141–52. [PubMed: 12110133]
9. Yadav D, Sarvetnick N. Cytokines and autoimmunity: redundancy defines their complex nature. *Curr Opin Immunol* 2003;15:697–703. [PubMed: 14630205]
10. Scrivo R, Di Franco M, Spadaro A, Valesini G. The immunology of rheumatoid arthritis. *Ann N Y Acad Sci* 2007;1108:312–22. [PubMed: 17893995]
11. Koch AE. The pathogenesis of rheumatoid arthritis. *Am J Orthop* 2007;36:5–8. [PubMed: 17694191]
12. Hochberg MC, Tracy JK, Hawkins-Holt M, Flores RH. Comparison of the efficacy of the tumour necrosis factor α blocking agents adalimumab, etanercept, and infliximab when added to methotrexate in patients with active rheumatoid arthritis. *Ann Rheum Dis* 2003;62:13–6.
13. Arend WP. Cytokine imbalance in the pathogenesis of rheumatoid arthritis: the role of interleukin-1 receptor antagonist. *Semin Arthritis Rheum* 2001;30:1–6. [PubMed: 11357165]
14. Burger D, Dayer JM, Palmer G, Gabay C. Is IL-1 a good therapeutic target in the treatment of arthritis? *Best Pract Res Clin Rheumatol* 2006;20:879–96. [PubMed: 16980212]
15. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003;421:744–8. [PubMed: 12610626]
16. Komiya Y, Nakae S, Matsuki T, Nambu A, Ishigame H, Kakuta S, et al. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2006;177:566–73. [PubMed: 16785554]
17. Vukkadapu SS, Belli JM, Ishii K, Jegga AG, Hutton JJ, Aronow BJ, et al. Dynamic interaction between T cell-mediated β -cell damage and β -cell repair in the run up to autoimmune diabetes of the NOD mouse. *Physiol Genomics* 2005;21:201–11. [PubMed: 15671250]
18. Mensah-Brown EP, Shahin A, Al-Shamisi M, Wei X, Lukic ML. IL-23 leads to diabetes induction after subdiabetogenic treatment with multiple low doses of streptozotocin. *Eur J Immunol* 2006;36:216–23. [PubMed: 16358360]
19. Luger D, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, et al. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med* 2008;205:799–810. [PubMed: 18391061]
20. Chabaud M, Fossiez F, Taupin JL, Miossec P. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J Immunol* 1998;161:409–14. [PubMed: 9647250]
21. Hirota K, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, et al. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* 2007;204:2803–12. [PubMed: 18025126]
22. McKenzie BS, Kastelein RA, Cua DJ. Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* 2006;27:17–23. [PubMed: 16290228]
23. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 2007;448:484–7. [PubMed: 17581588]
24. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179–89. [PubMed: 16473830]
25. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor- β induces development of the T(H)17 lineage. *Nature* 2006;441:231–4. [PubMed: 16648837]

26. Lubberts E, Joosten LA, Oppers B, van den Bersselaar L, Coenen-de Roo CJ, Kolls JK, et al. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *J Immunol* 2001;167:1004–13. [PubMed: 11441109]
27. Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y. 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 U S A* 2003;100:5986–90. [PubMed: 12721360]
28. Bush KA, Walker JS, Lee CS, Kirkham BW. Cytokine expression and synovial pathology in the initiation and spontaneous resolution phases of adjuvant arthritis: interleukin-17 expression is upregulated in early disease. *Clin Exp Immunol* 2001;123:487–95. [PubMed: 11298138]
29. Pearson CM. Development of arthritis, peri-arthritis and periostitis in rats given adjuvants. *Proc Soc Exp Biol Med* 1956;91:95–101. [PubMed: 13297719]
30. Szekanecz Z, Halloran MM, Volin MV, Woods JM, Strieter RM, Kenneth G, et al. Temporal expression of inflammatory cytokines and chemokines in rat adjuvant-induced arthritis. *Arthritis Rheum* 2000;43:1266–77. [PubMed: 10857785]
31. Kim EY, Chi HH, Bouziane M, Gaur A, Moudgil KD. Regulation of autoimmune arthritis by the pro-inflammatory cytokine interferon-gamma. *Clin Immunol* 2008;127:98–106. [PubMed: 18276192]
32. Kim EY, Chi HH, Rajaiah R, Moudgil KD. Exogenous tumor necrosis factor-alpha induces suppression of autoimmune arthritis. *Arthritis Res Ther* 2008;10:R38. [PubMed: 18380898]
33. Durai M, Kim HR, Bala K, Moudgil KD. T cells against the pathogenic and protective epitopes of heat-shock protein 65 are crossreactive and display functional similarity: novel aspect of regulation of autoimmune arthritis. *J Rheumatol* 2007;34:2134–43. [PubMed: 17937454]
34. Nakajima H, Takamori H, Hiyama Y, Tsukada W. The effect of treatment with recombinant gamma-interferon on adjuvant-induced arthritis in rats. *Agents Actions* 1991;34:63–5. [PubMed: 1793053]
35. Wiesenberger I, Van der Meide PH, Schellekens H, Alkan S. Suppression and augmentation of rat adjuvant arthritis with monoclonal anti-interferon-gamma antibody. *Clin Exp Immunol* 1989;78:245–9. [PubMed: 12412757]
36. Brasted M, Spargo LD, Mayrhofer G, Cleland LG. Blockade of IFN-gamma does not affect the arthritogenicity of T cells generated during the induction of adjuvant arthritis but exacerbates the polyarthritis produced by adoptive transfer of arthritogenic effector cells. *Immunol Cell Biol* 2005;83:189–95. [PubMed: 15748216]
37. Quintana FJ, Carmi P, Mor F, Cohen IR. Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J Immunol* 2002;169:3422–8. [PubMed: 12218165]
38. Paul AG, van Kooten PJ, van Eden W, van der Zee R. Highly autoproductive T cells specific for 60-kDa heat shock protein produce IL-4/IL-10 and IFN-gamma and are protective in adjuvant arthritis. *J Immunol* 2000;165:7270–7. [PubMed: 11120861]
39. Guedez YB, Whittington KB, Clayton JL, Joosten LA, van de Loo FA, van den Berg WB, et al. Genetic ablation of interferon-gamma up-regulates interleukin-1beta expression and enables the elicitation of collagen-induced arthritis in a nonsusceptible mouse strain. *Arthritis Rheum* 2001;44:2413–24. [PubMed: 11665984]
40. Manoury-Schwartz B, Chiocchia G, Bessis N, Abelsira-Amar O, Batteux F, Muller S, et al. High susceptibility to collagen-induced arthritis in mice lacking IFN-gamma receptors. *J Immunol* 1997;158:5501–6. [PubMed: 9164973]
41. Vermeire K, Heremans H, Vandeputte M, Huang S, Billiau A, Matthys P. Accelerated collagen-induced arthritis in IFN-gamma receptor-deficient mice. *J Immunol* 1997;158:5507–13. [PubMed: 9164974]
42. Chu CQ, Swart D, Alcorn D, Tocker J, Elkon KB. Interferon-gamma regulates susceptibility to collagen-induced arthritis through suppression of interleukin-17. *Arthritis Rheum* 2007;56:1145–51. [PubMed: 17393396]
43. Willenborg DO, Fordham SA, Staykova MA, Ramshaw IA, Cowden WB. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. *J Immunol* 1999;163:5278–86. [PubMed: 10553050]
44. Campbell IL, Oxbrow L, Harrison LC. Reduction in insulinitis following administration of IFN-gamma and TNF-alpha in the NOD mouse. *J Autoimmun* 1991;4:249–62. [PubMed: 1909136]

45. Jain R, Tartar DM, Gregg RK, Divekar RD, Bell JJ, Lee HH, et al. Innocuous IFN γ induced by adjuvant-free antigen restores normoglycemia in NOD mice through inhibition of IL-17 production. *J Exp Med* 2008;205:207–18. [PubMed: 18195074]
46. Tarrant TK, Silver PB, Wahlsten JL, Rizzo LV, Chan CC, Wiggert B, et al. Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J Exp Med* 1999;189:219–30. [PubMed: 9892605]
47. Tran EH, Prince EN, Owens T. IFN- γ shapes immune invasion of the central nervous system via regulation of chemokines. *J Immunol* 2000;164:2759–68. [PubMed: 10679118]
48. Furlan R, Brambilla E, Ruffini F, Poliani PL, Bergami A, Marconi PC, et al. Intrathecal delivery of IFN- γ protects C57BL/6 mice from chronic-progressive experimental autoimmune encephalomyelitis by increasing apoptosis of central nervous system-infiltrating lymphocytes. *J Immunol* 2001;167:1821–9. [PubMed: 11466408]
49. Fairweather D, Frisancho-Kiss S, Yung SA, Barrett MA, Davis SE, Gatewood SJ, et al. Interferon- γ protects against chronic viral myocarditis by reducing mast cell degranulation, fibrosis, and the profibrotic cytokines transforming growth factor- β 1, interleukin-1 β , and interleukin-4 in the heart. *Am J Pathol* 2004;165:1883–94. [PubMed: 15579433]
50. Gadina M, Hilton D, Johnston JA, Morinobu A, Lighvani A, Zhou YJ, et al. Signaling by type I and II cytokine receptors: ten years after. *Curr Opin Immunol* 2001;13:363–73. [PubMed: 11406370]
51. Matthys P, Vermeire K, Billiau A. Mac-1(+) myelopoiesis induced by CFA: a clue to the paradoxical effects of IFN- γ in autoimmune disease models. *Trends Immunol* 2001;22:367–71. [PubMed: 11429320]
52. Matthys P, Froyen G, Verdot L, Huang S, Sobis H, Van Damme J, et al. IFN- γ receptor-deficient mice are hypersensitive to the anti-CD3-induced cytokine release syndrome and thymocyte apoptosis. Protective role of endogenous nitric oxide. *J Immunol* 1995;155:3823–9. [PubMed: 7561088]
53. Duhe RJ, Evans GA, Erwin RA, Kirken RA, Cox GW, Farrar WL. Nitric oxide and thiol redox regulation of Janus kinase activity. *Proc Natl Acad Sci U S A* 1998;95:126–31. [PubMed: 9419340]
54. Okuda Y, Sakoda S, Fujimura H, Yanagihara T. Nitric oxide via an inducible isoform of nitric oxide synthase is a possible factor to eliminate inflammatory cells from the central nervous system of mice with experimental allergic encephalomyelitis. *J Neuroimmunol* 1997;73:107–16. [PubMed: 9058766]
55. Zhang X, Brunner T, Carter L, Dutton RW, Rogers P, Bradley L, et al. Unequal death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. *J Exp Med* 1997;185:1837–49. [PubMed: 9151709]
56. Qin HY, Chaturvedi P, Singh B. In vivo apoptosis of diabetogenic T cells in NOD mice by IFN- γ /TNF- α . *Int Immunol* 2004;16:1723–32. [PubMed: 15492021]
57. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004;4:762–74. [PubMed: 15459668]
58. Grohmann U, Fallarino F, Bianchi R, Orabona C, Vacca C, Fioretti MC, et al. A defect in tryptophan catabolism impairs tolerance in nonobese diabetic mice. *J Exp Med* 2003;198:153–60. [PubMed: 12835483]
59. Barrios-Rodiles M, Chadee K. Novel regulation of cyclooxygenase-2 expression and prostaglandin E2 production by IFN- γ in human macrophages. *J Immunol* 1998;161:2441–8. [PubMed: 9725242]
60. Chen X, Baumel M, Mannel DN, Howard OM, Oppenheim JJ. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4⁺CD25⁺ T regulatory cells. *J Immunol* 2007;179:154–61. [PubMed: 17579033]
61. Sheikh MS, Huang Y. Death receptor activation complexes: it takes two to activate TNF receptor 1. *Cell Cycle* 2003;2:550–2. [PubMed: 14504472]
62. Ben-Horin S, Bank I. The role of very late antigen-1 in immune-mediated inflammation. *Clin Immunol* 2004;113:119–29. [PubMed: 15451466]
63. La Cava A, Fang CJ, Singh RP, Ebling F, Hahn BH. Manipulation of immune regulation in systemic lupus erythematosus. *Autoimmun Rev* 2005;4:515–9. [PubMed: 16214088]

64. Campbell IK, O'Donnell K, Lawlor KE, Wicks IP. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. *J Clin Invest* 2001;107:1519–27. [PubMed: 11413159]
65. Mori L, Iselin S, De Libero G, Lesslauer W. Attenuation of collagen-induced arthritis in 55-kDa TNF receptor type 1 (TNFR1)-IgG1-treated and TNFR1-deficient mice. *J Immunol* 1996;157:3178–82. [PubMed: 8816431]
66. Tada Y, Ho A, Koarada S, Morito F, Ushiyama O, Suzuki N, et al. Collagen-induced arthritis in TNF receptor-1-deficient mice: TNF receptor-2 can modulate arthritis in the absence of TNF receptor-1. *Clin Immunol* 2001;99:325–33. [PubMed: 11358427]
67. Bush KA, Kirkham BW, Walker JS. The in vivo effects of tumour necrosis factor blockade on the early cell mediated immune events and syndrome expression in rat adjuvant arthritis. *Clin Exp Immunol* 2002;127:423–9. [PubMed: 11966757]
68. Eskandari F, Webster JI, Sternberg EM. Neural immune pathways and their connection to inflammatory diseases. *Arthritis Res Ther* 2003;5:251–65. [PubMed: 14680500]
69. Zakharova M, Ziegler HK. Paradoxical anti-inflammatory actions of TNF-alpha: inhibition of IL-12 and IL-23 via TNF receptor 1 in macrophages and dendritic cells. *J Immunol* 2005;175:5024–33. [PubMed: 16210605]
70. Butcher GA. Malaria and macrophage function in Africans: a possible link with autoimmune disease? *Med Hypotheses* 1996;47:97–100. [PubMed: 8869923]
71. Jacob CO, McDevitt HO. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 1988;331:356–8. [PubMed: 2893286]
72. Satoh J, Seino H, Abo T, Tanaka S, Shintani S, Ohta S, et al. Recombinant human tumor necrosis factor alpha suppresses autoimmune diabetes in nonobese diabetic mice. *J Clin Invest* 1989;84:1345–8. [PubMed: 2794065]
73. Jacob CO, Aiso S, Michie SA, McDevitt HO, Acha-Orbea H. Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): similarities between TNF-alpha and interleukin 1. *Proc Natl Acad Sci U S A* 1990;87:968–72. [PubMed: 2405400]
74. Grewal IS, Grewal KD, Wong FS, Picarella DE, Janeway CA Jr, Flavell RA. Local expression of transgene encoded TNF alpha in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J Exp Med* 1996;184:1963–74. [PubMed: 8920883]
75. Liu J, Marino MW, Wong G, Grail D, Dunn A, Bettadapura J, et al. TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat Med* 1998;4:78–83. [PubMed: 9427610]

Figure 1. A schematic model explaining the dual role of TNF- α and IFN- γ in the pathogenesis of AA

A specific threshold of TNF- α and IFN- γ is required for the initiation of autoimmune arthritis, but the secretion of these cytokines beyond a critical level triggers regulatory mechanisms that suppress the ongoing disease. The modulation of disease can be achieved either by direct injection of the pro-inflammatory cytokine (TNF- α / IFN- γ) or by immunization with a peptide/protein that activates T cells producing predominantly Th1 type pro-inflammatory cytokines. (AA= adjuvant arthritis; Mtb= *M. tuberculosis* H37Ra, heat-killed; R465= arthritis-protective epitope 465-479 of self (rat) hsp65.)