Commentary

Paradoxical roles of IFN-γ in models of Th1-mediated autoimmunity

Edward F Rosloniec[1,2,3], Kary Latham[3] and Yajaira B Guedez[2]

[1]VA Medical Center, Research Service (151), Memphis, Tennessee, USA
[2]Department of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, USA
[3]Department of Pathology, University of Tennessee Health Science Center, Memphis, Tennessee, USA

Corresponding author: Edward F Rosloniec (e-mail: erosloniec@utmem.edu)

Received: 13 May 2002 Revisions received: 3 July 2002 Accepted: 3 July 2002 Published: 17 July 2002

Arthritis Res 2002, **4**:333-336 (DOI 10.1186.ar432)
 (Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

T-cell responses to antigens are classified on the basis of the cytokines they produce as either Th1 (IFN-γ, IL-2) or Th2 (IL-4, IL-10), with these Th types being indicative of either cell-mediated or antibody-mediated responses, respectively. Using this classification, T-cell responses in MHC-class-II-restricted autoimmune diseases appear to be predominantly of the Th1 type, based on the presence of high levels of IFN-γ. This simplistic classification has recently been challenged, however, as disease incidence and severity are frequently elevated in animals that have a deficient IFN-γ response. The recent data discussed here indicate that the cytokine circuits involved in the regulation of cell-mediated and humoral immune responses during the development of autoimmune arthritis are more complex than originally proposed; perhaps our characterization of autoimmune responses as strictly Th1 or Th2 is overly simplistic, especially as it pertains to the role of IFN-γ.

Keywords: arthritis, autoimmunity, cytokines, IFN-γ

Introduction

Cytokines play critical roles in regulating the outcome of antigen-specific T-cell responses, and thus have been a major focus in the study of the pathogenesis of autoimmunity. On the basis of the original description by Mosmann *et al.* [1], we know that the cytokine profile of a T-cell response to an antigen is indicative of which T helper (Th) cell pathway is stimulated by the antigen-presenting cell. Th1 responses, generally characterized as cell-mediated immune responses, are identified primarily by the presence of IL-12, IL-2 and IFN-γ, whereas Th2 responses, generally characterized as humoral responses, are defined primarily by the production of IL-4 and IL-10. In addition to these characterizations, there is convincing evidence that these two pathways are antagonistic, in other words Th1 cytokines repress Th2 responses, and Th2 cytokines repress Th1 responses. Learning how to regulate these responses therapeutically, therefore, has become an important focus in autoimmunity research.

Most autoimmune diseases and models of autoimmunity in which susceptibility is associated with the expression of specific MHC class II allotypes appear to be of the Th1 type, based on these cytokine definitions of Th function. Thus considerable emphasis has been placed on developing means of altering the course of the autoimmune Th1 response to become that of a Th2 response, with the goal of downregulating the autoimmune pathogenesis. These cytokine networks not only influence the function of T cells involved in the pathogenesis of the autoimmune disease, however, but also affect qualitative differences in the antibody responses that are often associated with autoimmune disease, or, in some cases, represent the actual pathogenic mechanism. For example, IFN-γ, a cytokine strongly associated with a Th1 response, is an important regulator of the production of IgG2a antibody, a subclass frequently associated with a pathogenic autoantibody response, while IgG1 production (promoted by IL-4) predominates in a Th2 response. IFN-γ, therefore, has been

CIA = collagen-induced arthritis; IL = interleukin; IFN-γ = interferon γ; IFN-γR = interferon γ receptor; MHC = major histocompatibility complex; PG = proteoglycan; PGIA = proteoglycan-induced arthritis; R = receptor; RA = rheumatoid arthritis; Th = T helper cell.

Table 1

Role of IFN-γ in various models of autoimmunity.

Autoimmune model	Immunopathogenic mechanism	Genetic deletion	Effect on disease incidence or severity	References
Experimental autoimmune encephalomyelitis	T cells	IFN-γ	Increased	[10]
Experimental autoimmune myasthenia gravis	Antibody	IFN-γR	Decreased	[10]
Experimental autoimmune thyroiditis	T cells	IFN-γR	Accelerated, less severe	[5]
Collagen-induced arthritis	Antibody	IFN-γR & IFN-γ	Increased	[6–8]
Uveitis	T cells	IFN-γ	Increased	[4]
Proteoglycan-induced arthritis	T cells	IFN-γ	Decreased	[11]

considered a prime target for modulating autoimmunity, with the hypothesis being that if IFN-γ expression can be downregulated, then both the Th pathway and the production of pathogenic autoantibody can be altered. Data generated using models of autoimmunity have revealed a much more complex role of the Th1 cytokine IFN-γ in autoimmune pathogenesis than expected. As we discuss below for several models of autoimmunity, IFN-γ, despite being a component of a 'pathogenic' Th1 response, can also play a protective role in the development of an autoimmune response. Furthermore, the regulation of IgG2a and IgG1 isotypes appears to be more complex than previously thought.

IFN-γ and models of autoimmunity

The vast majority of autoimmune diseases studied and their corresponding animal models have been characterized as being mediated by the Th1 pathway, based on cytokine expression patterns in lymphoid and targeted tissues. Although the original paradigm of Th1 and Th2 cell function described a distinct separation of cytokines and function in T-cell-dependent immune responses, through the use of a number of models of autoimmunity it has become clear that these stereotyped roles do not accurately reflect the complexities of the biological immune responses. These complexities are perhaps best demonstrated in the analysis of the role of IFN-γ in models of autoimmunity. Early attempts to define the role of IFN-γ in autoimmunity by the administration of IFN-γ or neutralizing antibodies specific for IFN-γ yielded conflicting results. In some experimental systems disease was accelerated, while in others it was prevented, with these differences being confounded by a number of experimental variables. The use of models in which either IFN-γ or the IFN-γ receptor (IFN-γR) had been genetically deleted revealed that, in the absence of a competent IFN-γ response, disease incidence and severity were enhanced in the majority of autoimmune models tested (Table 1). These data include both models in which T cells are the primary mechanism of pathogenesis [2–4] as well as those in which autoantibody is the primary pathogenic mechanism [5–8]. Yet perhaps even more puzzling is that, for at least two models with seemingly different pathogenic mechanisms (experimental myasthenia gravis [9,10] and proteoglycan-induced arthritis [11]), expression of the experimental disease was found to be dependent on IFN-γ.

IFN-γ in collagen-induced autoimmune arthritis

One model of autoimmunity in which the role of IFN-γ has been studied in detail is collagen-induced arthritis (CIA) in the mouse. In this model, an autoimmune arthritis resembling rheumatoid arthritis (RA) is induced by immunization of genetically susceptible strains of mice with type II collagen [12]. Disease susceptibility is restricted by the murine class II molecule I-A (specifically I-A^r and I-A^q haplotypes) [13], and subsequently, $CD4^+$ T cells play a central role in the immunopathogenesis of this experimental autoimmune disease. The collagen type II (CII)-specific T-cell response is predominated by Th1 cells producing IL-2 and IFN-γ that in turn drive the production of complement-fixing CII-specific IgG2a, a major component in the pathogenesis of this experimental disease [14].

Early attempts to define the role of IFN-γ in CIA by the administration of IFN-γ or neutralizing antibodies specific for IFN-γ yielded conflicting results [15–19], probably because of variations in timing, sites and means of administration. Studies of CIA development in genetically susceptible mice in which IFN-γ (Y Guedez and E Rosloniec, unpublished observations) or the IFN-γ receptor (IFN-γR) [6,7] had been deleted revealed that autoimmune arthritis develops faster and is more severe in the absence of an IFN-γ response. Despite efforts by numerous investigators, a clear consensus on how the absence of a potent Th1 cytokine such as IFN-γ renders an animal more susceptible to a Th1-mediated autoimmune response is still lacking. Although it might be predicted that, in the absence of IFN-γ, there would be a compensatory increase in Th2 cytokines, such as IL-4 and IL-10, no evidence for enhanced expression of any of the Th2

cytokines in these models has been demonstrated. Thus, despite the association of CIA with a strong Th1 response, the absence of an IFN-γ response in genetically susceptible mice enhances the development of autoimmune arthritis, and this occurs despite the lack of CII-specific IgG2a that has been presumed to be a major factor in the initiation of the pathogenesis.

Analogous to the paradoxical role of IFN-γ in CIA is the apparent surprising role of its counterpart, IL-4. When the function of IL-4 was neutralized either by antibody administration or genetic deletion, the onset and severity of CIA were greatly reduced [20]. Similar results were obtained in a complementary approach using DBA/1 mice expressing an IL-2Rb/IL-4R chimeric transgene. In this approach, IL-2 binding of the receptor transmits a signal via the IL-4 pathway [21]. Like the IFN-γ-deficient mice, arthritis developed in these chimeric transgenic mice at an accelerated rate and with increased severity. The autoimmune disease was associated with an increase in type 2 cytokines (IL-4, IL-5, IL-10), and an increase in CII-specific IgG1 levels, with IgG2a levels comparable to those in nontransgenic mice. Despite the elevated levels of Th2 cytokines, however, IFN-γ production was not significantly affected, again indicating the complex relationships among these mediators.

A regulatory role of IFN-γ in models of autoimmune arthritis is also supported by studies using strains genetically nonsusceptible to CIA. Although CIA susceptibility is restricted to strains expressing H-2^q and H-2^r class II alleles, other strains, such as C57BL/6 (B6, H-2^b), develop marginal T-cell and B-cell immune responses to CII without developing autoimmune arthritis [8,13]. Yet when IFN-γ is genetically deleted from the B6 genome (B6 IFN-$\gamma^{-/-}$), these mice become acutely susceptible to the development of CIA [8,20]. The arthritis in the B6 IFN-$\gamma^{-/-}$ mice is accompanied by an enhanced T-cell response and high amounts of IgG1 and IgG2b CII-specific antibody. Like the studies in the CIA susceptible models, cytokine analysis did not reveal any significant changes in the remaining Th1 or Th2 cytokines but did reveal elevated levels of IL-1β in the lymph nodes and synovial cells of arthritic B6 IFN-$\gamma^{-/-}$ mice. The elevated levels of IL-1β appear to be important for the development of the disease, as treatment of B6 IFN-$\gamma^{-/-}$ mice with anti-IL-1β significantly reduced the incidence and the severity of the arthritis [8]. In all, these data serve as a clear example of the complexity of both the dynamics of the cytokine milieu as well as the complex relationships that exist between the Th1 and Th2 cytokines regulating the development of an autoimmune and inflammatory response.

Role of IFN-γ in proteoglycan-induced arthritis

Recently, Kaplan *et al.* [11] examined the role of IFN-γ in another model of autoimmune arthritis, proteoglycan-induced arthritis (PGIA). Like CIA, the induction of PGIA is based upon Th1-mediated cross-reactive immune responses between the heterologous immunogen (proteoglycan) and the self-antigen located in the articular joints [22–24]. The arthritis in PGIA is characterized by a progressive disease course with intermittent exacerbations and remissions reminiscent of the clinical appearance of RA. To date, only Balb/c mice have been found to be susceptible to PGIA [22–24], which is interesting in that this strain has a genetic predisposition to generating Th2 responses [25]. Although PGIA is considered a Th1-mediated experimental disease, it is clear that the immunopathogenesis involves a complex pattern of Th1 and Th2 cytokines with elevated levels of PG-specific IgG1 dominating in comparison to IgG2a, yet a strong predominance of IFN-γ over IL-4 in inflamed paws [11,26].

Despite the fact that both CIA and PGIA are considered to be Th1 models of RA, the role of IFN-γ appears to be totally different in these two models. Based on the recent report by Kaplan *et al.* [11], Balb/c mice genetically deficient in IFN-γ (knockout) are resistant to the development of PGIA. Arthritis incidence and severity were both found to be reduced in these mice in comparison to wild-type Balb/c mice, and, as would be expected, the amount of PG-specific IgG2a was also significantly decreased in the IFN-γ-deficient mice. Thus these data indicate that IgG2a is likely to be a major factor in the pathogenesis of this model, despite the observation that IgG1 predominates in the immune response to PG. These data were supported by studies of PGIA in IL-4-deficient Balb/c mice [11]. In the absence of IL-4, Balb/c mice developed an accelerated and very severe PGIA that was accompanied by an increase in IFN-γ and a sixfold increase in PG-specific IgG2a. Surprisingly, the levels of PG-specific IgG1 were only minimally decreased in the IL-4-deficient mice, suggesting that, at least in Balb/c mice, IgG1 production may be heavily influenced by yet other cytokines.

Conclusion

Animal models of autoimmune disease are providing a valuable means of analyzing the functional roles of cytokines in the pathogenesis of autoimmunity. The original description of Th1 and Th2 responses has provided us with a valuable framework for advancing our understanding of pathogenic T cell responses, and we now are beginning to understand the complexities that regulate these responses. Although the data from Kaplan *et al.* [11] provide some interesting insight into the development of a pathogenic autoimmune response, they represent single time point analyses, making it difficult to decipher the complex regulation of the humoral responses and the complex dynamics of the cytokine milieu present during the development of autoimmunity. Multiple time point analyses of antibody production and multiplexed cytokine expression profiling may help to increase our understanding of the complexities of the regulation of autoimmune

humoral responses and the role they play in mediating susceptibility to autoimmune disease. For example, mice treated with IL-18 or IL-18 plus IL-12 produced markedly more collagen-specific IgG1 and IgG2a than did controls, whereas IL-12 treatment alone enhances only the IgG2a responses [27]. Regardless, it is clear from these data and the studies from many others using murine models of autoimmunity that cytokine circuits involved in the regulation of humoral and cell-mediated immune responses in the development of autoimmune diseases are more complex than originally proposed, and perhaps our characterization of autoimmune responses as Th1 or Th2 is overly simplistic, especially as it pertains to the role of IFN-γ.

Acknowledgements

The work of the authors was supported in part by grants from the Department of Veterans Affairs, Memphis, TN, USA and by US Public Health Service Grants AR45201, AR47379, AR39166 from the National Institute for Arthritis and Musculoskeletal Diseases.

References

1. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: **Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins.** *J Immunol* 1986, **136:**2348-2357.
2. Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman CG: **Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE).** *J Immunol* 1996, **156:**5-7.
3. Caspi RR, Chan CC, Grubbs BG, Silver PB, Wiggert B, Parsa CF, Bahmanyar S, Billiau A, Heremans H: **Endogenous systemic IFN-gamma has a protective role against ocular autoimmunity in mice.** *J Immunol* 1994, **152:**890-899.
4. Jones LS, Rizzo LV, Agarwal RK, Tarrant TK, Chan CC, Wiggert B, Caspi RR: **IFN-gamma-deficient mice develop experimental autoimmune uveitis in the.** *J Immunol* 1997, **158:**5997-6005.
5. Alimi E, Huang S, Brazillet MP, Charreire J: **Experimental autoimmune thyroiditis (EAT) in mice lacking the IFN-gamma receptor gene.** *Eur J Immunol* 1998, **28:**201-208.
6. 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:**5507-5513.
7. Manoury-Schwartz B, Chiocchia G, Bessis N, Abehsira-Amar O, Batteux F, Muller S, Huang S, Boissier MC, Fournier C: **High susceptibility to collagen-induced arthritis in mice lacking IFN-gamma receptors.** *J Immunol* 1997, **158:**5501-5506.
8. Guedez YB, Whittington KB, Clayton JL, Joosten LA, van de Loo FA, van den Berg WB, Rosloniec EF: **Genetic ablation of interferon-gamma up-regulates interleukin-1beta expression and enables the elicitation of collagen-induced arthritis in a non-susceptible mouse strain.** *Arthritis Rheum* 2001, **44:**2413-2424.
9. Zhang GX, Xiao BG, Bai XF, Orn A, van der Meide PH, Link H: **IFN-gamma is required to induce experimental autoimmune myasthenia gravis.** *Ann NY Acad Sci* 1998, **841:**576-579.
10. Zhang GX, Xiao BG, Bai XF, van der Meide PH, Orn A, Link H: **Mice with IFN-gamma receptor deficiency are less susceptible to experimental autoimmune myasthenia gravis.** *J Immunol* 1999, **162:**3775-3781.
11. Kaplan C, Valdez JC, Chandrasekaran R, Eibel H, Mikecz K, Glant TT, Finnegan A: **Th1 and Th2 cytokines regulate proteoglycan-specific autoantibody isotypes and arthritis.** *Arthritis Res* 2002, **4:**54-58.
12. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B: **Immunisation against heterologous type II collagen induces arthritis in mice.** *Nature* 1980, **283:**666-668.
13. Wooley PH, Luthra HS, Stuart JM, David CS: **Type II collagen induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates.** *J Exp Med* 1981, **154:**688-700.
14. Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM: **Induction of arthritis with monoclonal antibodies to collagen.** *J Immunol* 1992, **148:**2103-2108.
15. Mauritz NJ, Holmdahl R, Jonsson R, Van der Meide PH, Scheynius A, Klareskog L: **Treatment with gamma-interferon triggers the onset of collagen arthritis in mice.** *Arthritis Rheum* 1988, **31:**1297-1304.
16. Nakajima H, Takamori H, Hiyama Y, Tsukada W: **The effect of treatment with interferon-gamma on type II collagen-induced arthritis.** *Clin Exp Immunol* 1990, **81:**441-445.
17. Boissier MC, Chiocchia G, Bessis N, Hajnal J, Garotta G, Nicoletti F, Fournier C: **Biphasic effect of interferon-gamma in murine collagen-induced arthritis.** *Eur J Immunol* 1995, **25:**1184-1190.
18. Cooper SM, Sriram S, Ranges GE: **Suppression of murine collagen-induced arthritis with monoclonal anti-Ia antibodies and augmentation with IFN- gamma.** *J Immunol* 1988, **141:**1958-1962.
19. Williams RO, Williams DG, Feldmann M, Maini RN: **Increased limb involvement in murine collagen-induced arthritis following treatment with anti-interferon-gamma.** *Clin Exp Immunol* 1993, **92:**323-327.
20. Ortmann RA, Shevach EM: **Susceptibility to collagen-induced arthritis: cytokine-mediated regulation.** *Clin Immunol* 2001, **98:**109-118.
21. Chen Y, Rosloniec E, Goral MI, Boothby M, Chen J: **Redirection of T cell effector function in vivo and enhanced collagen-induced arthritis mediated by an IL-2R beta/IL-4R alpha chimeric cytokine receptor transgene.** *J Immunol* 2001, **166:**4163-4169.
22. Finnegan A, Mikecz K, Tao P, Glant TT: **Proteoglycan (aggrecan)-induced arthritis in BALB/c mice is a Th1-type disease regulated by Th2 cytokines.** *J Immunol* 1999, **163:**5383-5390.
23. Glant TT, Cs-Szabo G, Nagase H, Jacobs JJ, Mikecz K: **Progressive polyarthritis induced in BALB/c mice by aggrecan from normal and osteoarthritic human cartilage.** *Arthritis Rheum* 1998, **41:**1007-1018.
24. Glant TT, Mikecz K, Arzoumanian A, Poole AR: **Proteoglycan-induced arthritis in BALB/c mice. Clinical features and histopathology.** *Arthritis Rheum* 1987, **30:**201-212.
25. Reiner SL, Locksley RM: **The regulation of immunity to Leishmania major.** *Annu Rev Immunol* 1995, **13:**151-177.
26. Hollo K, Glant TT, Garzo M, Finnegan A, Mikecz K, Buzas E: **Complex pattern of Th1 and Th2 activation with a preferential increase of autoreactive Th1 cells in BALB/c mice with proteoglycan (aggrecan)-induced arthritis.** *Clin Exp Immunol* 2000, **120:**167-173.
27. Leung BP, McInnes IB, Esfandiari E, Wei XQ, Liew FY: **Combined effects of IL-12 and IL-18 on the induction of collagen-induced arthritis.** *J Immunol* 2000, **164:**6495-6502.

Correspondence

Dr Edward F Rosloniec, VA Medical Center, Research Service (151), 1030 Jefferson Avenue, Memphis, TN 38104, USA. Tel: +1 (901) 577-7281; fax: +1 (901) 577-7273; e-mail: erosloniec@utmem.edu