Editorial

A Bridge Between Interferon-α and Tumor Necrosis Factor in Lupus

In this issue of *The Journal*, investigators from the University of Oviedo, Spain, report novel insights on the function of antimalarials and expand their data on the influence of polymorphisms regulating tumor necrosis factor (TNF) and interleukin 10 (IL-10) expression[1]. The authors show that among patients with systemic lupus erythematosus (SLE) treated with antimalarials, those who express the genotype associated with low TNF and high IL-10 levels had significantly higher serum interferon-α (IFN-α) versus those with other genotypes, while those with the genotype associated with high TNF and low IL-10 levels had increased regulatory T cells. TNF and IL-10 promoter polymorphisms did not differentiate patients based on levels of IFN-α or regulatory T cells among those not treated with antimalarials. These data stimulate consideration of the mechanisms by which chloroquine and hydroxychloroquine modulate cytokine expression in SLE, and suggest approaches that may eventually allow prediction of who will respond to antimalarials, a mainstay of SLE therapy.

In addition, however, this report may provide one pillar of a bridge to span a perceived divide in lupus cytokine studies: the one between IFN-α and TNF. Both cytokines play essential roles in host defense against microbes, but their roles in autoimmune and inflammatory diseases are complex and not yet understood. One proposal addressing this complexity suggested that immunity can be seen as a dynamic system driven by opposite vectors, with SLE developing when IFN-α dominates and rheumatoid arthritis developing when TNF is the dominant cytokine in a pathologic process[2]. In reality, however, there is solid evidence that both IFN-α and TNF are overexpressed in many patients with SLE, and that, indeed, both may be highly relevant in the disease process. Since our groups have worked on either side of the divide, we appreciate the opportunity to discuss the mechanisms that account for contributions of both IFN-α and TNF and to reframe SLE as a disease that calls for a more nuanced view that incorporates complex regulatory circuits and potential roles for both cytokines as potential therapeutic targets. It is clear that this bridge cannot be completed with today's limited understanding of these circuits. However, the data presented by Patricia Lopez and colleagues may help us to sketch its structure.

Although somewhat hampered by a lack of simple detection methods of sufficient specificity, and thus based on labor intensive biological assays, IFN-α serum levels have long been known to be increased in SLE, and to correlate with lupus disease activity[3-5]. More recently, microarray and real-time polymerase chain reaction analysis of SLE peripheral blood cells showed increased expression of many IFN-inducible genes, demonstrating a "signature" consistent with *in vivo* stimulation by IFN-α, again in association with disease activity[6-8]. The source of IFN-α in SLE has been elucidated in that plasmacytoid dendritic cells of SLE patients were found to produce increased amounts of IFN-α[9], and increased IFN-α expression in the skin has been demonstrated[10]. IFN-α is known to have diverse effects on immune function, and many of those activities, including maturation of dendritic cells and induction of proinflammatory chemokines, are consistent with observations of altered immune function in patients with SLE[11,12].

Further support for the hypothesis that IFN-α plays a pathophysiologic role in SLE comes from observations that recombinant human IFN-α, when administered for hepatitis or other diseases, can induce SLE flares and drug-induced lupus[13]. Finally, preliminary data from a first clinical trial of a monoclonal anti-IFN-α antibody in SLE showed ablation of the IFN signature and suggested clinical improvement in at least some study subjects[14].

TNF is similarly highly increased in SLE and correlates well with SLE disease activity[15,16]. Although soluble TNF receptors are also significantly increased in patient serum, TNF bioactivity is not inhibited by availability of those

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receptors[17]. TNF is also overexpressed in active lupus nephritis, where again it correlates with disease activity[16], and has been found in lupus skin lesions[18]. Monocytes/macrophages are presumed to be the major cellular source of TNF in lupus and can produce TNF when stimulated with immune complexes[16]. Indeed, immune complexes are important stimuli for both IFN-α and TNF. In the case of IFN-α, immune complexes containing nucleic acids can access intracellular Toll-like receptors after binding to Fc receptors and induce IFN-α. Fc receptors are also likely to contribute to TNF production induced by lupus immune complexes.

Consideration of the relative roles of IFN-α and TNF in the pathogenesis of SLE has been complicated by the occasional development of lupus autoantibodies when those cytokine pathways are manipulated. Lupus autoantibodies and occasionally clinical disease have developed in patients who receive therapeutic recombinant IFN-α, demonstrating the capacity of that cytokine to promote autoimmunity in a susceptible host[19]. In the case of TNF, however, it is the blockade of this cytokine that is associated with induction of antichromatin (and antiphospholipid) autoantibodies and the occasional induction of transient drug-induced lupus-like syndromes[20]. The immunologic basis of these clinical observations has not been fully elucidated. The data regarding IFN-α are generally felt to be consistent with the concept that the cytokine promotes autoimmunity through multiple mechanisms, while several hypotheses have been raised to explain the capacity of TNF blockade to promote autoantibody production. These include induction of apoptosis, particularly by infliximab, as well as modulation of other immunoregulatory properties of TNF[16]. The idea that loss of an inhibitory effect of TNF on IFN-α production might contribute to the production of lupus autoantibodies has also been considered, based on studies demonstrating the suppression of IFN-α production by TNF[21]. However, while serum IFN-α levels can increase under some conditions of TNF blockade[22], there is no experimental support for the concept that those increases account for autoantibody production.

While the hypothesis that there are IFN-α-driven diseases such as SLE and TNF-driven diseases such as RA[11] presents a model that serves to simplify understanding of rheumatic diseases, a consideration of the stages of disease pathogenesis as well as levels of disease activity suggests a more complex picture. We propose a role for both these cytokines in the pathogenesis of SLE as the scope of the immune complex triggers of cytokine induction broaden and the extent of inflammation and tissue damage grow over time (Figure 1). The first clinical studies with the combination of azathioprine with the anti-TNF antibody infliximab suggested potential benefits on organ inflammation, whereas no flares were observed, despite a transient increase in antichromatin and antiphospholipid autoantibodies[23,24].

What does the new report add to this complex picture? On the one hand, the authors have investigated both IFN-α and TNF serum levels of SLE patients, a combination that had so far not been published. Although one could criticize the assays used, their results are probably correct, since the increased levels of IFN-α and TNF are consistent with the previous literature. In contrast to what some may have expected, serum levels of IFN-α and TNF in both SLE patients and healthy individuals showed a weak positive correlation ($r = 0.275$). Additional new data measuring IFN-α functional activity and TNF by ELISA in SLE define patterns of cytokine expression based on a genetic variant in PTPN22, a phosphatase that regulates lymphocyte activation[25]. Patients with the SLE-risk allele tend to show either a correlation of IFN-α and TNF values or an IFN-α high pattern. Unfortunately, neither the study of Lopez, *et al* nor the new study of PTPN22 variants provides data on the disease activity or organ involvement of the SLE patients. Preliminary data from one of our groups indicate that SLE patients can be characterized based on peripheral blood gene expression profiles into those expressing the IFN signature, those expressing activation of an inflammation pathway (including TNF, IL-1, and IL-8 mRNA), and those with both or neither pathway activated (Kirou KA, Crow MK, unpublished observations). Consistent with a concomitant role for both IFN-α and TNF in lupus nephritis, those patients with gene expression profiles showing activation of both pathways had a higher prevalence of renal disease than those with either pathway activated. It is tempting to speculate that another novel finding, that TNF induces low level IFN-β production in monocytes/macrophages[26], may play a role in the dual activation of these cytokine pathways, since both of these type I interferons use the same receptor.

The other novel piece of evidence lies in the discrepancy between patients genetically prone to produce high levels of TNF (TNFhi) and those not carrying such TNFhi alleles, but producing high levels of IL-10 (TNFlo IL-10hi)[1]. Serum IFN-α levels of the latter, TNFlo IL-10hi patients, were almost twice as high taking antimalarials versus non-users of these drugs. In contrast, this was not true for the TNFhi patient population, where the IFN-α levels of those taking antimalarials were no different than those taking other agents.

To our eyes, these findings look like a threshold effect of TNF on IFN-α (Figure 2). Under conditions in which both cytokines are present at low levels, each would help to keep the other in check. But in the setting of immune system activation, most likely triggered by immune complexes in SLE, but potentially as well by activation of Toll-like receptors by pathogens, this reciprocal downmodulation is overridden, and production of both cytokines is increased. If TNF is reduced below a certain threshold, however, as effected by TNF blockade[22], by antimalarials in TNFlo individuals[1], or when TNF is genetically absent in lupus-prone New

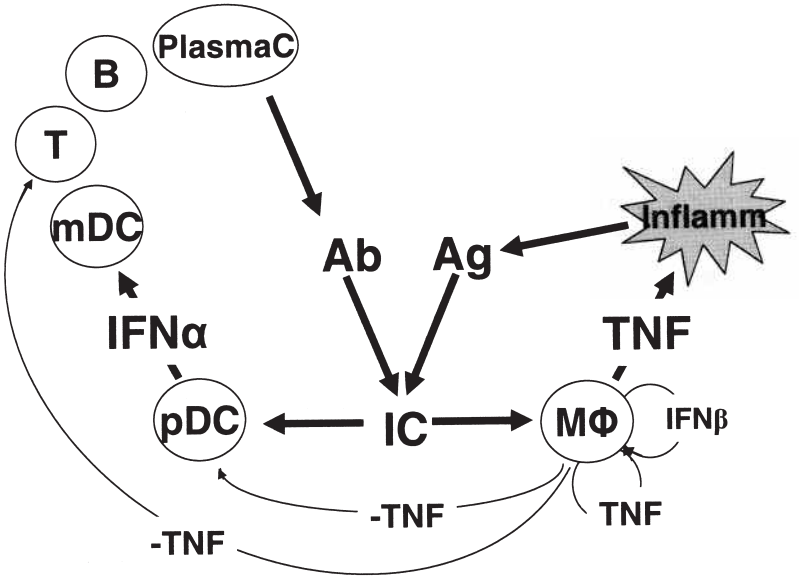

Figure 1. Overview of the role of TNF and IFN-α and their interplay in SLE.

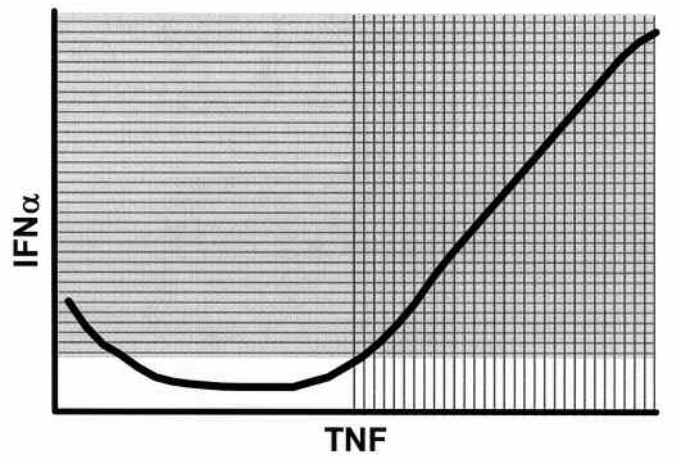

Figure 2. Putative relationship between TNF (x-axis) and IFN-α (y-axis) serum levels in SLE. Vertical stripes denote pathologically increased TNF, horizontal stripes increased IFN-α.

Zealand White x New Zealand Black mice[27], IFN-α production is unchecked and therefore increases, with the potential to influence mechanisms of tolerance and autoimmunity.

In line with the data presented by Lopez, *et al*[1], evidence is accumulating that genetic factors are significantly influencing the pathophysiology of SLE, probably including the involved cytokine patterns. As noted above, a PTPN22 variant associated with SLE is associated with higher IFN-α activity, and patients genetically prone to produce more TNF are at an increased risk of developing the disease. It will take a truly comprehensive overview to explain the interplay between various genetic background motifs, environmental factors, and immunoregulatory circuits that result in distinct patterns of cytokine production, organ involvement, and levels of disease activity. While the picture describing these complex scenarios is not yet clear, we may already have most of the puzzle pieces we will need to bring clarity to the role of cytokines in SLE.

For the moment, it appears that in well controlled lupus and in health, both IFN-α and TNF will control each other. On the other hand, when TNF is diminished below a certain threshold, such as by TNF blockers[24] or by antimalarials in TNFlo SLE patients[1], IFN-α production may be unleashed, increasing the propensity to develop autoantibodies. Once those autoantibodies are available, augmented innate immune system activation by autoantibody-containing immune complexes can perturb cytokine networks to favor IFN-α, TNF, or both. Immune complexes can induce TNF production, particularly in individuals carrying TNFhi alleles. Similarly, nucleic acid-containing immune complexes, including DNA-anti-dsDNA and RNA-anti-RNA-binding protein immune complexes, will induce plasmacytoid dendritic cells and probably other cell types to produce IFN-α. Accordingly, as autoimmunity broadens in a lupus patient, the level of cytokines will increase, along with SLE disease activity, in line with the finding of Lopez, *et al* that serum levels of TNF and IFN-α correlate[1]. In addition, TNF may directly stimulate the interferon pathway by inducing IFN-β [26]. New understanding of the interplay of multiple gene variants, together with improved knowledge of the distinct events involved in initiation of autoimmunity, amplification of immune dysregulation, and activation of effector mechanisms that implement target organ damage will be needed to complete the full picture.

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