Do cytokines play a role in systemic lupus erythematosus?

ABSTRACT—Excessive production of pathogenic autoantibodies is one of the hallmarks of systemic lupus erythematosus (SLE). The mechanisms that underlie this excessive production are still unclear. Although there is considerable evidence to suggest that both T-cells and B-cells play an important role in the aetiology of SLE, convincing abnormalities at the T-cell receptor or immunoglobulin gene loci have not been demonstrated. In this regard, because cytokines play a pivotal role in the inflammatory response, a defect in the immunoregulation of B-cells by cytokines should be considered as a potential contender in disease aetiology. The hypothesis proposed here is that multiple cytokine-mediated defects are present in individuals with lupus and that both cytokine production and the response of B-cells to cytokines may be defective.

Autoantibodies with specificity for a variety of self-antigens are one of the hallmarks of SLE. These autoantibodies persist at high titre and form immune deposits in various organs, including the kidney [1,2]. While the deposition of immune complexes and the recruitment of other pro-inflammatory mediators can explain the mechanism of the injury, the causes that underlie high-level autoantibody expression are not well understood. One hypothesis that can be formulated is that the potential to generate lupus-causing autoantibodies may not be unique to lupus-prone individuals; such antibodies may represent normal components of the immune system that, under physiologic conditions, remain unstimulated or suppressed. Their high-level expression in lupus may result from the abnormal response of B-cells to regulatory stimuli such as cytokines or from an appropriate B-cell response to abnormal levels of certain cytokines.

In SLE, inbred animal models with a consistent and spontaneous incidence of a lupus-like disease have provided invaluable information on disease pathogenesis [reviewed in ref. 15]. Three main types of lupus mice have been used: MRL/lpr-lpr mice, whose genetic derivation is known (LG 75%, AKR 12.6%, C3H 12.1%, and C57BL/6 0.3%); New Zealand mice, which are of unknown derivation; and BXSB mice (from C57BL/6 and SB/Le). In all these strains, the genetic background predisposes to a late-life disease that becomes clinically manifest and then fatal in the second year of life. Additionally, an accelerating factor, acting on the lupus-prone genetic background, can cause conversion to an early-life disease, clinically apparent in the first few months of life and fatal within five to seven months. Even though observations derived from inbred strains of mice need to be interpreted cautiously with regard to human disease, lupus mice do provide an insight into the role of genetic, environmental, and regulatory factors that may be important in disease.

Although the notion that regulatory stimuli may underlie the pathogenesis of lupus is appealing, experimental data to support it have been relatively sparse. Most attention has been focused on finding a structural or functional abnormality in the complex mechanisms generating the antibody repertoire. Several investigators have looked for abnormal Ig germ line or T-cell receptor genes, the formation of unusual variable, diversity, and/or joining (V,D,J) gene segment rearrangements or excessive somatic mutation.

The importance of T-cells in the induction of lupus has been supported by cell transfer experiments [3–6] and by the ameliorating effects of administered anti-CD4 antibodies [7–11]. In studies on the role of TcR germ line genes, no difference between lupus and normal strains has been detected at the C α, C β, V α, or V β loci [12–14]. The expressed T-cell receptor repertoire and tolerance-related V β clonal elimination phenomena have also been examined and no defect found [15].

Several investigators have studied the contribution of abnormalities in immunoglobulin genes to the development of lupus. No VH germ line or Igh-VH and V kappa haplotype associations with autoantibody production and lupus disease have been detected [16–20]; nor is there a defect in Ig switch or in Igh enhancer regions [21]. The role of somatic mutation has been extensively investigated [22–24] and also appears to be an unlikely cause for the high level of autoantibody production seen in lupus.

Collectively, these studies suggest that the Ig and T-cell receptor locus is probably normal in lupus. Recently, we and others have developed a technique that uses pairs of highly specific oligonucleotide probes hybridised at high stringency to study the presence and expression of a single V gene in the immune repertoire of autoimmune and normal individuals [25,26]. This has been a significant advance because previously the complexity of large V gene families made it difficult to detect individual V genes. We have

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identified a germ line gene (MRL 330) that is repeatedly used to encode for pathogenic anti-DNA antibodies in lupus mice and is present but poorly expressed in some normal mice. This suggests that the Ig repertoire may be one of the genetic risk factors in lupus. The presence of the MRL 330 gene in some but not all normal mice also strongly suggests that the regulation of potentially pathogenic V genes in B-cells by cytokines may be of great relevance. The influence of cytokines on the activation of these B-cells, in general, can be via a directed flow of lymphokines from the T-cell to the B-cell and the modulating influences of other cytokines in the extracellular milieu, derived chiefly from macrophages and T-cells.

Most attention has been paid to IL-2, IL-1, and TNF. Investigators have studied IL-2 levels in lupus mice and the administration of IL-2 *in vivo*. IL-2 levels in autoimmune mice show an age-related decline which correlates with disease activity [27]. Experiments with IL-2 gene therapy in MRL/lpr-lpr mice also support an aetiological role for IL-2 [28]. However, not all studies concur. Strom and colleagues [29] show that an IL-2 receptor antibody administered to NZB/W lupus mice results in significant amelioration of disease activity, whereas Owen *et al* found that IL-2 treatment caused neither an improvement nor an acceleration in disease activity [30].

Two groups in particular have studied IL-1 and TNF in detail [31–34]. Again, their results are in conflict. Although both groups looked at the expression of IL-1 and TNF in macrophages, Kelley *et al* found enhanced TNF and IL-1 expression in glomerular macrophages from MRL/lpr mice [31,32], while Beller's group found defective IL-1 and normal TNF gene expression in peritoneal macrophages in several autoimmune murine strains [33,34]. This difference in cytokine expression between peritoneal and glomerular macrophages has not been explained.

The studies with IL-2, IL-1, and TNF suggest that multiple cytokine defects are probably important in the pathogenesis of lupus. This contention is supported by the presence of abnormal levels of other cytokines [35–37]. However, given the interactions of different cytokines, it is also conceivable that the abnormal production of one or two cytokines may initiate the release of a cascade of other cytokines that amplify the effect of the initiating stimulus; abnormal levels of several cytokines involved in this cascade would then be an epiphenomenon.

The evidence for a direct effect of cytokines on B-cells in lupus individuals is restricted to *in vitro* experiments. Klinman *et al* [35] observed that NZB/W B-cells incubated with IL-4 have a defect in their ability to undergo class switching and to proliferate after switching. In MRL/lpr-lpr mice, however, they found that the response to IL-4 was similar to controls but that the age-related change in isotype from IgM to IgG in MRL/lpr-lpr mice was the result of increased production of IFN gamma. Other investigators have found that there may also be defects in the responsiveness of B-cells to normal levels of cytokines [37,38].

These experimental observations point to a complex role for cytokines in the regulation of B-cell production of autoreactive immunoglobulin. In NZB/W mice, there may be altered responsiveness to normal levels of cytokines, whereas in MRL/lpr-lpr mice there appears to be an appropriate B-cell response to increased levels of cytokines. Moreover, the synergistic or antagonistic effect of one cytokine on another may add a further level of complexity to their overall role in lupus. Nevertheless, the hypothesis that cytokine regulation of B-cells may be a central defect in the pathogenesis of lupus does appear tenable. More detailed study in *in vitro* and *in vivo* settings with a panel of cytokines administered individually or in combination may elucidate the nature of the cytokine defect more fully.

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