Cytokines and Anxiety in Systemic Lupus Erythematosus (SLE) Patients Not Receiving Antidepressant Medication

A Little-explored Frontier and Some of Its Brief History

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Autoimmunity travelled from the theoretical roots planted by Burnet with the clonal selection theory and the early finding of a mouse providing a test for the role of the thymus as a source of forbidden clones. This chapter briefly reviews early work with the NZB mouse and presents results of an analysis of associations between cytokines and physical and psychometric parameters in systemic lupus erythematosus (SLE) patients not medicated with antidepressants. Some cytokines, particularly IFN-γ, relate significantly to physical symptoms and anxiety. We conclude with the speculation that anxiety is linked to innate immunity and more severe neuropsychiatric disease in SLE to adaptive immunity.

Key words: **clonal selection; SLE; cytokines; NZB mice; anxiety; autoantibodies; depression**

Introduction: Old Frontiers and Explorers

The development of a simple test that enabled the detection of antibodies against red blood cells by Coombs in the 1940s[1] became an extremely valuable tool in the diagnosis of autoimmune disease in humans and in the first experimental model of spontaneous systemic lupus erythematosus (SLE)—the New Zealand Black (NZB) mouse.[2] The NZB mouse became, in turn, an extremely valuable tool for testing Burnet's clonal selection theory of antibody production.[3] According to the biographical memoir in the Australian Academy

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of Science,[4] of his contributions to immunology, Burnet, with Dr. Margaret Holmes,[5] devoted the last few years of his life at the bench exploring various aspects of the biology and immunopathology of these mice, which spontaneously develop a high incidence of haemolytic anaemia of an autoimmune type, at an early age, and other signs recalling human systemic lupus erythematosus.[2] Having shown that the anaemia could be transferred to young isologous mice by transfer of spleen cells from older mice Burnet and Holmes showed that the affected mice developed characteristic thymic lesions."[5]

The appearance of germinal centers in several organs, including the thymus, was confirmed later by East, de Sousa, and Parrott (reviewed in Ref. 6). The notion that the thymus was the source of forbidden clones influenced the decision to thymectomize one case of severe SLE.[7] Two years later, East *et al*. showed that neonatal thymectomy in NZB mice had no effect on the development of the SLE-like pathology resulting in some cases in worsening of the condition.[8] Today, it has become evident that the benefits of thymectomy in autoimmune disease are questionable, and there are several reports of development of SLE in thymectomized myasthenia gravis patients.[9,10]

Why start this chapter with reference to such old, seemingly outdated frontiers in autoimmunity? They illustrate well the difference between tool and theory and the approach of the two old explorers—Coombs, whose contribution is still of practical usefulness today, and Burnet, whose theoretically inspired contributions in immunology led him to share the Nobel Prize for Physiology and Medicine with Sir Peter Medawar in 1960, and whose convictions without full experimental testing came to precipitous action on the part of surgeons. Analysis of the tissues of the thymectomized animals by one of us very soon showed that in some cases of partial thymectomy the animals actually developed more severe disease, indicating already then that the balance between thymus-derived cell populations might be much more complex than anticipated at the time. That of course proved to be the case.

The New Explorers

In the mid-1960s, the discoveries of the function of the thymus and of thymus-derived cell populations demonstrating the existence of a cellular in addition to the well-established humoral immunity built a relatively simple image of the immune system consisting of two main populations. T and B cells were shown to have different origins and distinct destinations in the peripheral lymphoid organs. This view of the immune system would absorb the attention of immunologists for years. It is to a certain extent shattered into many more puzzle pieces today as tools gained an exquisite degree of precision in the definition of cell populations within the T and B cell families. This degree of precision reached not just the cell surface marker level with the discovery of monoclonal antibodies[11] and technical achievements in fluorescent activating cell sorting, but—and of particular interest to this chapter—extended to the definition of activation markers preferentially associated with cell subsets and their respective products, the cytokines, at the cell and molecular levels.[12]

It can thus be said that larger-than-life figures like Coombs, Burnet, or Medawar are being replaced by an increasing array of tools acting themselves as explorers, building a cutting-edge frontier in a hypothesis-free world, where infection itself is diminishing but all roads lead to the clinic.

New Frontiers in Autoimmunity

The importance of situations in which the activation of the immune system occurs in the absence of the purpose of fighting an infection or rejecting an allograft cannot be underestimated. In general, however, one feels that other noninfectious diseases such as allergy, atherosclerosis, obesity, and the metabolic

syndrome are the focus of greater attention than autoimmunity. Nevertheless, the availability of disease models of spontaneous immune activation without infection, like SLE, enables us to redraw the cutting edge of the immune system itself from new actions of innate immunity products, the cytokines, as illustrated by the results of the study described in the next section.

The Present Study: A Little-explored Frontier

SLE continues to be a significant model of a "strictly immunological" disease. Our interest focused on the neuropsychiatric frontier. Depression and anxiety are reported in SLE and have been related to strong psychosocial influences or regarded as a result of the disease immunological dysfunction.

The results to be presented in this brief review refer only to 9 SLE nonmedicated female patients between the ages of 21 and 60. They are part of a larger study of SLE patients with and without depression, psychiatric patients with major and minor depression, and healthy controls within the same age range (20–65) in a total of 99 women who submitted to psychosocial evaluation. The evaluation consisted of a battery of tests that included, for diagnosis of major depression according to *DSM-IV* criteria, a semistructured interview, the Diagnostic Interview for Genetic Studies (DIGS).[13] The Mini Mental State Examination (MMSE) was used to assess basic cognitive measures in all subjects.[14] The Portuguese version of the original Short Form Health Survey (SF-36) was used to assess health-related quality of life.[15,16] The degree to which the participants appraised their lives as being stressful during the previous month was assessed using the 10-item version of the Perceived Stress Scale (PSS), providing a more subjective evaluation of stress than life events scores.[17] In order to evaluate current severity of depression and anxiety, the Hospital Anxiety and Depression Scale (HADS) was used.[18] Affective State evaluation was done using the PANAS (Positive and Negative Affect Schedule).[19] Clinical indices of disease were based on SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) and SLLIC/ACR (Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index). Determination of cytokines was done for IFN-γ, IL-6, IL-8, IL-10, and TNF-α, in peripheral blood by flow cytometry using a Cytomics FC500 flow cytometer (Beckman-Coulter, Brea, CA) and a BMS710FF human Th1/Th2 10plex Kit I (BenderMedSystems GmBH, Vienna, Austria). Cytokine levels, as expected, were higher in the SLE patients than in controls (Table 1). The differences reached statistical significance in IFN-γ, IL-6, and IL-5.

As shown in Figure 1, negative correlations were seen between IFN-γ and Role Function Physical, Role Function Emotional, Social Function and Mental Health in the 9 SLE patients without major depression and without medication (Fig. 1). Similar negative correlations were observed with IL-8 (data not shown).

One most interesting result was the finding of a clear separation between the positive correlation of IFN-γ and IL8 (not shown) with anxiety, but not with depression (Fig. 2). To our knowledge this is the first demonstration of an association between IFN-γ levels and anxiety in an immune activation disease. Furthermore, it is of some interest to have found that IL-8 is the second cytokine to relate to an equal number of measures of psychological function. The results also showed that TNF-α followed with negative correlations with Physical Function, Mental Health and positively with Anxiety (data not shown) in SLE patients without depression. Finally, IL-6 correlated significantly with Emotional Function and IL-10 with Physical Function, indicating that subtle differences may exist between the cytokine targets (data not shown). No correlations were found between psychosocial measures and cytokine levels in depressed patients taking medication and in controls.

Interestingly, a trend was detected of higher cytokine levels in SLE patients with major

TABLE 1. Cytokine Levels in a Group of 9 Patients Without Depression Who Are Not Taking Antidepressants or Corticoids Confirm Immune Activation Nature of SLE

Group	IFN-γ[a]	IL-1β	IL-2	IL-6[a]	TNF-α	TNF-β	IL-8	IL-4	IL-5[a]	IL-10
SLE	129.2[b]	134.3	138.1	108.1	183.8	118.1	127.6	169	158.1	157
($n = 9$)	± 63.7	± 66.9	± 70.3	± 41.1	± 168.7	± 47.5	± 58.7	± 91.9	± 80.2	± 103
Controls	79.6	93.3	82	75.4	104.3	78.7	84.7	108.9	93.7	88.9
($n = 31$)	± 53.2	± 61.5	± 48.9	± 37.6	± 85.5	± 43.2	± 49.1	± 94.6	± 58.2	± 55

[a]$P < 0.05$; [b]Mean ± Standard deviation

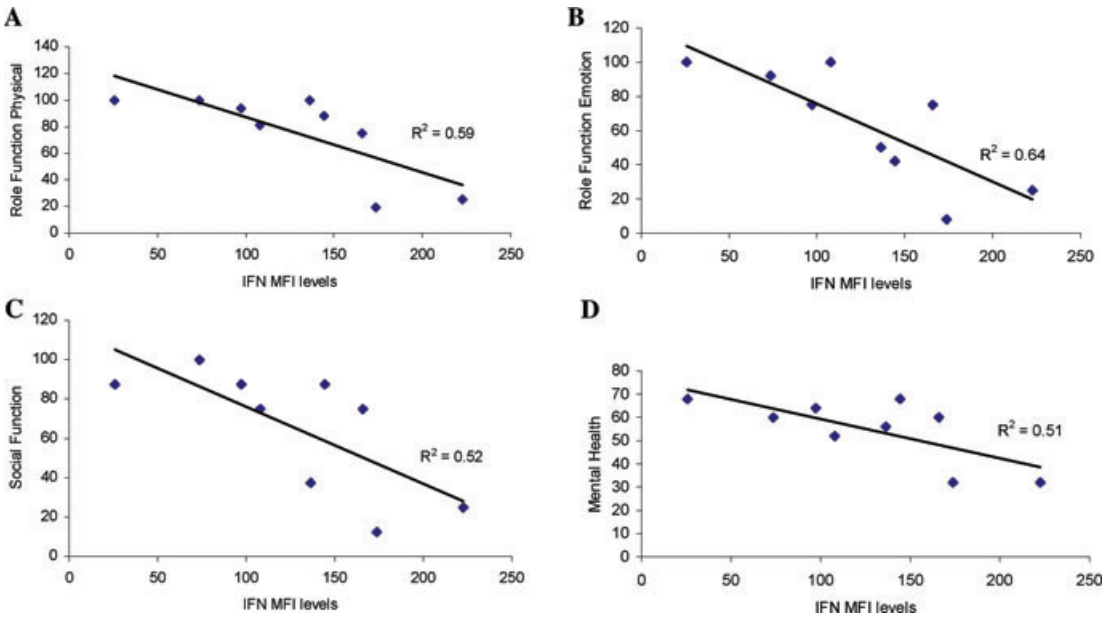

Figure 1. (**A–D**) Negative correlations between IFN-γ and Role Function Physical, Role Function Emotional, Social Function and Mental Health.

depression when medicated with antidepressants, statistically significantly different in IL-8 ($P = .010$), IL-10 ($P = .017$), and TNF-α ($P = .018$), when compared to non-antidepressant medicated patients of the same group (nonparametric Mann-Whitney test). The effectiveness of antidepressants has been vividly questioned recently as a "myth based on a thousand clinical trials."[20]

The interactions seen between cytokine expression and HADS A scores indicate that the immune system through some cytokines may have a role in the pathophysiology of anxiety in SLE, in the absence of psychiatric disorder.

Our results support further the results of other studies with ovarian cancer patients and burn-out school teachers in which pro- and anti-inflammatory cytokine levels have been directly linked to the development of anxiety.[21,22]

Where Old and New Explorers Meet

The novel contribution of the results reported here is the significant association of cytokine levels with anxiety in SLE patients without major depression and not receiving medication. In 1996 Schrott and Crnic had compared the behavior of hybrids of the original NZB mouse first studied by Burnet—the NZBx NZW F1 (B/W) hybrid and NZW mice—that do not develop autoimmune disease: "B/W mice were less active at both

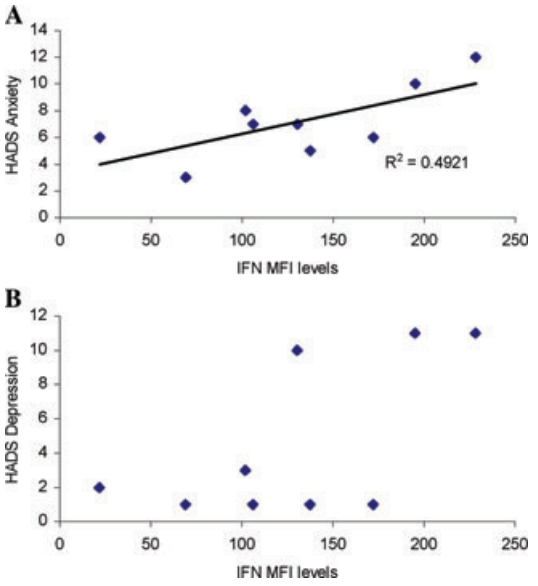

Figure 2. (**A, B**) Positive correlations between IFN-γ and anxiety and no correlation with depression in SLE-NMD patients without medication.

ages, suggesting a genetic component to this behavioral difference. Anxiety behavior and exploratory behavior did not differ between B/W and NZW mice at 6 weeks; however, at 12 weeks B/W mice displayed more anxiety behavior and less exploratory behavior, indicating that these behaviors were related with the development of autoimmune disease. Prior experience with these tasks increased anxiety behavior in B/W but not NZW mice, suggesting that B/W mice may be more sensitive to anxiogenic experiences."[23]

Later, to confirm the relationship between disease progression and development of behavioral abnormalities, Schrott and Crnic[24] subjected B/W and nonautoimmune NZW mice to the chronic treatment with a soluble IFN-γ receptor (sIFN-γ R), a treatment known to retard autoimmune disease progression, or vehicle, beginning at 6 weeks of age: "After 6 weeks of treatment, elevated plus maze and novel object testing revealed that although sIFN-γ R treated B/W mice still differed from NZW mice, chronic sIFN-γ R treatment significantly retarded the development of behavioral abnormalities in the B/W mice, while the NZW mice were not affected by this treatment. sIFN-γ R treated B/W mice were more active in both the plus maze and novel object tasks, and displayed less plus maze anxiety behavior and more exploratory activity in the novel object task compared to vehicle treated B/W mice." The authors concluded in 1998 that "the results added to the growing evidence that lupus-associated behavioral abnormalities are a direct effect of the autoimmune disease."[24]

Concluding Remarks

The present chapter illustrates well how SLE as an established autoimmune disease provides an excellent experimental and clinical model for testing in the clinic today what was cutting-edge observation in experimental animals yesterday. The frontiers between these two worlds are now being pushed by cytokines as in the 1960s they were pushed by theories trying to explain self-tolerance. At both ends of the rainbow of time, the thymus, thymus-derived populations and cytokines seem unquestionably critical to the development of the autoimmune disease and of anxiety.

We wish to conclude with a "cutting-edge speculation." Others studying SLE have shown an association of antibodies directed against N-methyl-D-aspartate receptor subunit NR2 (anti-NR2) in cerebrospinal fluid (CSF) of patients with neuropsychiatric manifestations in systemic lupus erythematosus.[25] The speculation consists of proposing that cytokines as the expression of innate immunity are associated with anxiety, and that autoantibodies as the expression of adaptive immunity are associated with more severe neuropsychiatric symptoms, including depression.

It is fortunate that one of us has been at both ends of the rainbow to witness and be part of the progress of knowledge in this field. It feels slow, but it seems steady. It makes one wonder if immune-based therapies should not be extended from cutting-edge experimental findings to the clinic, and vice versa—if

cutting-edge findings in the clinic should not be more thoroughly tested experimentally.

Conflicts of Interest

The authors declare no conflicts of interest.

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