Abstract
Systemic lupus erythematosus (SLE or lupus) is a systemic autoimmune disease characterized by a constellation of varied clinical presentations, although the nearly universal presence of autoantibodies is a salient unifying feature. Ongoing research efforts focus on understanding the complex combination of genetic and environmental factors that lead to SLE in select individuals. Our previous work has demonstrated that years before diagnosis abnormal autoantibody responses are present in the sera of patients who will subsequently develop lupus and, further, that the initial targets of two of these key responses (anti-Sm B’ and anti-60 kD Ro alone) have been identified for some patients. Indeed, our results suggest that the first lupus-specific autoantibodies arise from particular antibodies directed against Epstein-Barr virus Nuclear Antigen-1 (EBNA-1) and that infection with Epstein-Barr virus (EBV) is an environmental risk factor for lupus. The predicted sequence of events is normal immunity, followed by Epstein-Barr virus infection, the generation of anti-EBNA-1 antibodies, then followed by those particular anti-EBNA-1 antibodies that also bind lupus-specific autoantigens (Sm or Ro), followed by the development of more complex autoimmune responses, and, finally, culminating in clinical disease. Studies from others and those underway suggest that lupus patients have unusual immune responses to Epstein-Barr virus. In aggregate, these results are consistent with an immune response against Epstein-Barr virus being important in at least some patients for the initiation of lupus autoimmunity.

While modern medical research has provided extraordinary insights into the causation and pathophysiology of many infectious, genetic, metabolic, and malignant diseases, the fundamental environmental origins for a number of inflammatory disorders are notably unknown. Systemic lupus erythematosus (SLE or lupus) was among these, but the picture is becoming clearer for this disorder. While autoantibodies are recognized as important in diagnosis and in pathogenic mechanisms that generate specific clinical manifestations, the basis for their generation remains a subject of speculation and controversy.

All lupus patients appear to have autoantibodies of one type or another. As defined by the antigens that they bind, more than 50 known specificities must exist. Some are particular to lupus (eg, anti-Sm, anti-ribosomal P, anti-double stranded DNA), while others are also found in other rheumatologic conditions (eg, antinuclear antibodies [ANA], anti-single stranded DNA, rheumatoid factor, anti-phospholipid, anti-Ro (SS-A), anti-La (SS-B), and anti-nuclear ribonucleoprotein (anti-nRNP).

Development of Autoantibodies in SLE
What is the relationship between autoantibodies and disease onset? Recent data1-4 show that most lupus-related autoantibodies precede the clinical manifestations of lupus by years. Indeed, a ranking of lupus autoantibody specificities exists, with some preceding lupus diagnosis by a mean of more than 3 years. These include antinuclear antibodies, anti-Ro, anti-La, and anti-phospholipid antibodies. Anti-double stranded DNA is intermediate...
Initial Targets of Select Lupus Autoantibodies

In a small number of lupus patients, particular autoimmune responses have been observed as they develop. In the situations with anti-Sm and anti-Ro (without anti-La) these observations have provided powerful insights into the origins of lupus autoimmunity. Of the cases evaluated to date that have developed anti-Sm B under observation, the autoimmune progression appears to be provoked by the same antigenic epitope in every anti-Sm positive patient. The available data are consistent with PPPGM-RPP (single amino acid code for Pro-Pro-Pro-Gly-Met-Arg-Pro-Pro) being the initial structure recognized by autoantibodies. The next structure is PGMRGP, then RGAPPPGMGP, and finally PGRRGPP.\textsuperscript{4} Beyond this point, the antigenic epitopes bound by autoantibody from anti-Sm patients vary among many structures and do not follow an obvious pattern. This degree of fidelity in fine specificity as the immune response matures is almost without precedent in outbred mammalian immune responses to complex protein antigens. Whether or not the consistent progression in specificity will be modified by the observed development of anti-Sm in subsequent subjects is anticipated with great interest.

The anti-60 kD Ro response (when present alone, meaning without its frequently associated anti-La response) is similar in that a single epitope, TKYKQRNGWSHKD, appears to initiate anti-Ro autoantibody formation in all of the anti-Ro-positive lupus patients so far studied.\textsuperscript{3} In contrast to the maturation of the anti-Sm response, patients do not develop antibodies binding the same second Ro epitope. Rather, the autoimmune response spreads to a number of different second epitopes in these individuals, thereby exhibiting more variation than the response in patients with the early anti-Sm autoantibody response.

The critical structure that defines the onset of autoimmunity must be the initial lupus autoantigen epitope recognized by the immune system. Since our appreciation of the cellular interactions leading to lupus in man are so rudimentary, our studies are focused on the much more accessible humoral immune recognition events. Stored sera are repositories of preserved antibody repertoires at a fixed point in time. No comparable or accessible way to evaluate T cell or antigen presentation at a particular point in the past currently exists.

In approaching the pathogenesis of lupus from the perspective of humoral immunity, the critical step to investigate is the generation of the first lupus-specific autoimmune response. Sera are available from a collection of 130 SLE patients who had serial stored serum samples from before lupus diagnosis. Many of these individuals have initial samples that contain no autoantibodies.\textsuperscript{2} These future patients have no autoantibodies, not even low levels of the humoral lupus specificities. Therefore, the abnormal autoimmune responses appear to be acquired and not persistent from germ line antibodies that may have been present since infancy.

This is not to argue that lupus could not be genetic nor that the progression of events we observe could not be the stepwise progression of a predetermined program, genetic or otherwise. The questions are, when does the first lupus-specific autoimmune response occur and does a critical antigen provoke it? If true, then what is the nature of this substance?

Since we have observed a transition from absence to presence of lupus-specific autoantibodies in several patients, and since lupus is usually acquired in adulthood after an otherwise immunologically normal life,
we presume that most if not all lupus patients experience a period of normal immunity without the presence of lupus-specific autoimmune responses, as presented in Figure 1.

Parsimony, as a principle in scientific thinking, argues that the simplest explanation is the most defensible and, hence, preferable. We know that as many as 90 structures (epitopes) of the spliceosome protein autoantigens (Sm and nRNP) can be recognized by autoantibody (in both man and animal models).\(^6,7\) While more than 20 epitopes are found in the 60 kD Ro system.\(^3,8\) We cannot imagine a mechanism that would allow all of this complexity to burst from the immune humoral repertoire simultaneously. Further, the available evidence argues against such a bizarre scenario. Indeed, the existent data and argument of parsimony support the transition into humoral autoimmunity beginning with a single structure. Once provoked from its slumber by this autoantigenic structure into responding, the autoimmune response expands to involve other structures of the autoantigen, a process known as “B cell epitope spreading” (reviewed in Harley and James\(^6\) and Schlomchik et al\(^8\)).

Two related important implications follow from the imputed existence of the “first autoimmune epitope” (Fig. 2). First, only this initial structure must be closely tied to an immune history that precedes lupus autoimmunity. The first epitope, to the extent that it is a single, unique peptide sequence, therefore is the foundation from which to peer into the immunologic past to understand how those particular autoantibodies binding that first epitope arose. Both the anti-Sm and anti-Ro systems appear to develop from single, unique epitopes, PPPGMRPP and TKYKQRNGWSHKD, respectively, both of which appear capable of initiating lupus autoimmunity.\(^4,5\) They would be predicted to originate from a single B cell that has generated an antibody with the requisite properties (Fig. 2).

Second, we postulate that the recognition of all of the lupus-specific autoimmune epitopes that follow recognition of the first structure is dependent upon the first epitope. The subsequent responses could not occur if the autoimmune recognition of that first structure had not provided the immunologic basis for the responses that followed. This is an implication that directly follows from there being a unique first structure. The fact that no other first structures have yet been identified further support this position though there would be no present theoretical reason that all patients with a particular autoantibody initiate their responses against that specificity beginning with exactly that structure.

In addition, what we now know about immune regulation teaches us that once immune recognition of the antigen by antibody has occurred, then pathways and processes are available for immune processing and autoantigen presentation that did not exist previously for that antigen in that individual. The autoantigen presentation that occurs as a consequence of antibodies binding to the first structure is an attractive mechanism to generate the subsequent complexity of these autoimmune responses. This mechanism for generating an autoimmune response is sometimes referred to as “Immune Ignorance.”

Those first or primordial lupus autoantibodies binding PPPGMRPP and TKYKQRNGWSHKD must either arise directly from the germline or indirectly, through a regulated immune maturation process (or processes) that occurs during the course of life. The most obvious candidate source would be a particular predisposing heteroimmune antibody response and, within it, a particular specificity. This would be the heteroimmune response primordial to the lupus autoimmune response. The sequences of the autoantibodies that appear to initiate lupus autoimmunity are not currently available, however, and the question of whether they are germline sequences has not been directly addressed. The observation that the first lupus autoantibodies arise in the midst of a complex heteroimmune response against EBNA-1 (see below) would be more consistent with their being generated from the mutated DNA template for high affinity, mature antibodies, but this prediction has also yet to be directly tested.

Potential Origins of Initial Autoimmune Responses

The first critical hint of the origin of lupus autoimmunity came from the sequence PPPGMRPP. This is the first epitope of Sm B to be bound by autoantibody and defines

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Figure 2 Progression of a heteroimmune anti-EBNA-1 response to an autoimmune anti-Sm response. This schematic representation presents the postulated critical moment when the first cross-reactive autoantibody appears. Before autoimmunity the anti-EBNA-1 response is exclusively directed against EBNA-1 (Left Panel). The transitional or cross-reactive antibody binds both EBNA-1 and self (Top Center Panel). This is the first autoimmune autoantibody. Later autoantibodies form that bind only self (eg, Sm or Ro) (Right Panel) and do not bind EBNA-1.
The data in both the Sm and Ro examples presented confirm by an association of lupus with viral Epstein-Barr virus (with an odds ratio of ~50) has been infection. Indeed, in children a serologic association of lupus would be associated with Epstein-Barr virus in experimental animals. These results were the proof of principle that immune recognition of a single epitope was sufficient to initiate anti-Sm autoimmunity. Further, they are also consistent with the possibility that some limited-specificity lupus humoral autoimmunity arises from anti-Epstein-Barr virus humoral heteroimmunity.

To further test the potential generality that lupus autoimmunity may arise from the anti-Epstein-Barr virus heteroimmunity and taking a hint from PPPGRPP of anti-Sm, we tested whether the first autoantibodies binding 60 kD Ro at TKYKQRNGWSHKD also bound Epstein-Barr virus Nuclear Antigen-1 (EBNA-1). Again, a cross-reaction was found within EBNA-1, but this time with a structure, GGSGSGPRHRDGVR, that has no primary amino acid sequence homology with Ro. Immunization with either TKYKQRNGWSHKD or GGSGSGPRHRDGVR induced specific anti-Ro autoantibodies and anti-EBNA-1 antibodies in experimental animals.

The data in both the Sm and Ro examples presented above are consistent with a cross-reactive antibody being critically important in the induction of lupus autoimmunity (Fig. 2). This antibody has 2 (or more) identical binding sites, either of which will specifically bind either EBNA-1 or the self-antigen (eg, Sm or Ro). Of course, for the Sm and Ro systems, different cross-reactive antibodies are expected to mediate this critical step. For Sm that antibody is expected to bind PPPGRPP (of Sm) and PPPGRPRPP (of EBNA-1) for Ro that antibody binds TKYKQRNGWSHKD (of Ro) and GGSGSGPRHRDGVR (of EBNA-1). We do not know whether or not the relative paucity of EBNA-1, which has an abundance that must be many, many orders of magnitude below Sm or Ro in living human tissues, allows affinity maturation to occur against the self-antigen while maintaining high anti-EBNA-1 affinity. This mechanism, however, has some obviously attractive features.

**Association of Epstein-Barr Virus With SLE**

If two of the major autoimmune specificities arise from the anti-EBNA-1 response, then it is little wonder that lupus would be associated with Epstein-Barr virus infection. Indeed, in children a serologic association of Epstein-Barr virus (with an odds ratio of ~50) has been confirmed by an association of lupus with viral Epstein-Barr virus DNA from the peripheral blood. In addition, a large study comparing 196 adult SLE patients and 392 matched controls likewise found association of previous Epstein-Barr virus exposure with SLE. No consistent associations between SLE and other common herpes viruses, such as cytomegalovirus, herpes simplex 1, or herpes simplex 2, were found in these studies. While some studies have failed to detect this association, the predominating evidence favors association of lupus with Epstein-Barr virus infection, particularly when technical issues in the serologic and viral DNA detection assays are considered (for more detailed reviews please see McClain et al and James et al).

Because such a large proportion of the human population is infected with Epstein-Barr virus, an association of lupus with Epstein-Barr virus could explain only a small part of the risk of developing lupus, albeit a crucial small part. Calculating relative risk based on the assumption that Epstein-Barr virus causes lupus provides a risk of only ~1.05 when lupus patients (99% infected by Epstein-Barr virus) are compared to normal controls (~95% infected by Epstein-Barr virus). In other words, the epidemiologic impact of Epstein-Barr virus infection is small. If, for example, lupus occurs in <1:1000 adult women, then this requirement would remove only the ~50 of them that were not Epstein-Barr virus infected, leaving just one that does develop lupus, and she is not in this way distinguished from the ~950 that never do.

**Epstein-Barr Virus–specific Differences in SLE**

Three major possibilities are apparent to explain the greater part of the risk of lupus beyond Epstein-Barr virus association. First, stochastic processes may play a role. We already know that lupus is concordant in only 20% to 40% of identical twins. Environmental factors and random events may intercede to prevent lupus in up to three quarters or so of the twin lupus cases who would otherwise have been affected, as measured from the perspective of the twin with lupus. Alternatively, additional factors beyond Epstein-Barr virus and genetics may also be required for the complete phenotype of SLE to develop.

Second, variation in the virus could explain the different clinical consequences. While ample evidence for viral variation within normal populations and select malignancies has been published (reviewed in Griffin), no evidence that lupus occurs in concentrated foci within select populations exists. In addition, recent studies have shown no increased incidence of specific viral strain or viral strain coinfection in SLE patients compared to controls.

Third, perhaps risk of lupus is derived from the variations of the host response against Epstein-Barr virus. This possibility is consistent with the idea that dysregulated immune responses are the important risk factors for lupus. If they particularly involve EBNA-1,
then the existing data may be construed to support this proposition.

Indeed, EBNA-1 is the obvious point of departure to explore the immune response against Epstein-Barr virus. Antibodies against the first epitope in both anti-Sm and anti-Ro bind to EBNA-1, consistent with the idea that lupus autoimmunity originates in the anti-EBNA-1 humoral immune response. In addition, EBNA-1 has the unusual property of inhibiting its own antigen presentation, causing an absence of CD8 T cell recognition in most normal individuals.

Recent data show that a higher proportion of pediatric lupus patients have anti-EBNA-1 antibodies than do Epstein-Barr virus infected normal controls. In addition, the fine specificities of the anti-EBNA-1 antibodies formed in lupus patients are distinguishable from those formed in normal individuals. In contrast, neither anti-EBNA-2 nor anti-EI-CMV (early immediate antigen of cytomegalovirus) antibodies have different fine specificities when lupus patients are compared to controls. SLE patients have also been found to have aberrant T cell responses, with suggestions of increased interferon-producing CD4-positive Epstein-Barr virus-specific cells. Dysfunctional CD8 responses in SLE patients compared to controls have also been observed (reviewed in more detail in James et al).

Conclusions

In conclusion, the data are consistent with a model for the generation of lupus autoimmunity with six discernable proposed steps (Fig. 3). First, an immune repertoire develops normally in an individual who otherwise has a propensity for lupus autoimmunity. Second, such an individual is infected by Epstein-Barr virus. Third, anti-EBNA-1 antibodies result. Fourth, she or he generates the lupus pattern of anti-EBNA-1 antibodies and, perhaps, benign autoimmunity. Fifth, anti-EBNA-1 antibodies are generated that cross-react with the first epitope of Ro or Sm. This is the central and critical step that defines the onset of lupus-specific autoimmunity (for anti-Sm and anti-Ro, in any case) that we predict first occurs in a single B cell. Sixth, epitope spreading occurs, possibly along with pathogenic autoimmunity, and the process culminates in clinical illness. The generality of this model has yet to be established, and undiscovered components of risk are virtually certain to remain to be described. In the meantime, this hypothesis for the origin of lupus autoimmunity appears to explain the data now available. Our challenge now is to modify and extend this level of understanding...or to disprove it.

References

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