

Photosensitivity Diseases: Cutaneous Lupus Erythematosus

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Ultraviolet radiation plays an important role in the induction of lesions in many patients with cutaneous lupus. In the photosensitive subset of lupus, subacute cutaneous lupus, the effects of ultraviolet radiation likely act in concert with specific autoantibodies, particularly anti-Ro-related autoantibodies, to produce lesions. Potential effects of ultraviolet radiation on the induction of cutaneous lupus, and the potential interplay of specific autoantibodies with ultraviolet radiation are

discussed. The steps involved in the induction of cutaneous lupus lesions by ultraviolet radiation have not been fully elucidated. Recent advances in phototesting and analysis of the genetics of lupus should clarify the events leading to photosensitive cutaneous lupus lesions. Key words: photosensitivity diseases/lupus erythematosus/ultraviolet radiation/anti-Ro antibodies. Journal of Investigative Dermatology Symposium Proceedings 4:73-78, 1999

Lupus erythematosus (LE) has long been observed to be a photosensitive disease (Smith and Cyr, 1988); however, the term "photosensitivity" is poorly defined, even though it is a criterion for the classification of systemic LE (Tan *et al*, 1982). Photosensitivity may mean decreased minimal erythema dose, a sensation of burning of the skin, exacerbation of systemic symptoms, or induction of cutaneous lesions upon exposure of the skin to the sun. A recent study documents the poor correlation between a personal history of photosensitivity and a decreased minimal erythema dose (Doria *et al*, 1996). Another report indicates a frequent concurrence of transient lesions of polymorphous light eruption in LE patients, confounding the evaluation of the symptom of photosensitivity (Hasan *et al*, 1997). In this review, the term "photosensitivity" will be used to indicate induction or exacerbation of cutaneous LE lesions as a result of sun exposure.

Not all LE patients exhibit photosensitivity, and not all cutaneous manifestations are related to sun exposure. For example, lupus panniculitis and livedo reticularis are not notably affected by the sun. Even within a category of skin disease, individual patients may respond differently to sun exposure. Discoid lupus (DLE) lesions may be initiated or exacerbated by sun exposure in some patients, but may apparently be unrelated to sun exposure in others (Prystowsky *et al*, 1975; Lee *et al*, 1994b). The subset of cutaneous LE that is perhaps most clearly related to sun exposure is subacute cutaneous LE (SCLE), and it is on SCLE that this review will focus first. (For categorization and description of the types of lesions occurring in patients with lupus, see Sontheimer and Provost, 1996.)

SCLE: THE PROTOTYPE OF PHOTSENSITIVE CUTANEOUS LUPUS

SCLE was recognized as a distinctive subset of cutaneous LE by Gilliam *et al* in 1979 (Sontheimer *et al*, 1979). In essence, the same clinical phenotype was described by Provost *et al* in their reports of "ANA-negative" lupus (Provost *et al*, 1977; Maddison *et al*, 1981). Evidence for a role for ultraviolet (UV) exposure in the pathogenesis of SCLE comes from observations of patients that sun exposure results in lesion formation, the usual limitation of SCLE lesions to sun-exposed skin (although the mid-facial skin is characteristically and inexplicably spared), and the predilection for fair-skinned individuals with skin phenotype I or II (Sontheimer, 1989; David-Bajar *et al*, 1992). The observation that certain photosensitizing drugs, such as thiazide diuretics and sulfonylureas, can induce SCLE is perhaps also an indication that sun exposure plays a role in the pathogenesis (Reed *et al*, 1985). Finally, as will be discussed later, SCLE lesions can be reproduced by phototesting, although with more difficulty than expected (Lehmann *et al*, 1990).

Two very exciting observations confirmed the uniqueness of SCLE and provided insight regarding its likely pathogenesis. Sontheimer *et al* reported that SCLE is strongly associated with a particularly autoantibody specificity, anti-Ro (anti-SSA) (Sontheimer *et al*, 1982), and Weston *et al* reported that neonatal lupus (NLE) is strongly associated with anti-Ro (Weston *et al*, 1982). Because the skin disease of NLE is a photosensitive, non-scarring form of cutaneous LE, it appears to be the neonatal equivalent of adult SCLE (Lee, 1993). In NLE, the anti-Ro autoantibodies are produced in the mother and cross the placenta during pregnancy. The limitation of disease activity in cutaneous NLE to the first few months of life is a strong indicator of the likelihood that the maternal anti-Ro antibodies are causing, or at least contributing to, the disease.

The association of SCLE with anti-Ro explains why patients with SCLE lesions had previously been characterized as "ANA negative". Human anti-Ro autoantibodies do not always cross-react with mouse Ro antigen. In the past years, ANA testing was often performed on murine tissue, and in that type of assay antibodies to Ro may not be detected. Current ANA testing is generally performed on human cell substrates, which are a reliable means of detecting anti-Ro.

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Abbreviations: DLE, discoid lupus erythematosus; DDLE, disseminated discoid lupus erythematosus; LE, lupus erythematosus; LEP, lupus erythematosus panniculitis; NLE, neonatal lupus erythematosus; SCLE, subacute cutaneous lupus erythematosus; SLE, systemic lupus erythematosus; TLE, tumid lupus erythematosus.

Table I. Ro-associated antigens

Antigen	Molecular weight	Function or putative function
60 kDa Ro (Ro60, 60 kDa SSA)	60 kDa	? ribosome synthesis, assembly, or transport; ? salvage pathway for mutant rRNA precursors
52 kDa Ro (Ro52, 52 kDa SSA)	52 kDa	? transcription factor
La (SSB)	48 kDa	control of transcription involving RNA polymerase III
Calreticulin	46 kDa	calcium storage; numerous other functions and putative functions

Giving credence to the possibility that autoantibodies play a central role in the pathogenesis of lupus are recent studies of SLE where extracutaneous disease is reproduced in animals by infusion of anti-DNA antibodies or by immunization with the Sm autoantigen (Tsao *et al*, 1992; James *et al*, 1995). The anti-Ro autoantibody specificity has itself been directly implicated in animal models as a cause of the major extracutaneous manifestation of NLE, congenital heart block (Alexander *et al*, 1992; Garcia *et al*, 1994; Boutjdir *et al*, 1998; Miranda-Carus *et al*, 1998).

Given that autoantibodies are likely to be critically important to the understanding of SCLE and of photosensitivity in SCLE, the autoantibodies and autoantigens of SCLE will be described first before discussing potential links between the autoantibodies and photosensitivity.

THE AUTOANTIBODIES AND AUTOANTIGENS OF SCLE

Although the autoantibody specificity originally associated with SCLE is anti-Ro, a multiplicity of anti-Ro-related autoantibody specificities may be found in SCLE sera. The major specificities include anti-60 kDa Ro, anti-52 kDa Ro, anti-La, and anti-calreticulin (**Table I**) (Lee *et al*, 1994b; Sontheimer *et al*, 1995).

The originally described "Ro" antigen is a protein of 60 kDa that may be bound *in vivo* to one of four small RNA called "Y RNA" or "hY RNA" (human Y RNA) (Clark *et al*, 1969; Steitz *et al*, 1983). "Ro" represents the first two letters of the last name of the index patient from whom the autoantibodies were characterized. (The "La" and "Sm" antigens were named using the same convention.) An autoantigen characterized using autoantibodies from patients with Sjögren's syndrome and given the name "SSA" was shown to be identical to "Ro" (Alspaugh and Tan, 1975; Alspaugh and Maddison, 1979). The functions of 60 kDa Ro protein and its associated hY RNA are not established. It has been proposed that 60 kDa Ro functions in a salvage pathway for mutant rRNA precursors (O'Brien and Wolin, 1994). We have closely examined the cellular locations of 60 kDa Ro protein and hY RNA, and have proposed based on their presence in the nucleolar periphery and in ribosome-rich areas of the cytoplasm, that they may be involved in ribosome synthesis, assembly, or transport (Farris *et al*, 1997). We also noted that, although 60 kDa Ro protein may be in a complex with hY RNA, separate pools of the 60 kDa Ro protein and the Ro hY RNA exist within the cell.

Two decades after 60 kDa Ro was described, a second specificity common in anti-Ro-positive sera was discovered and the antigen identified as a 52 kDa protein (Ben-Chetrit *et al*, 1988). This protein was given the name 52 kDa SSA, or 52 kDa Ro. It is reasonable to expect that there is some relationship between 52 kDa Ro and 60 kDa Ro, because antibodies to both proteins frequently coexist, but the nature of that relationship is not entirely clear. There is no sequence homology between 60 kDa Ro and 52 kDa Ro, yet a cross-reactivity between native 60 kDa Ro and denatured 52 kDa Ro has been reported (Itoh *et al*, 1992). Another group, however, reported a lack of such cross-reactivity (Yell *et al*, 1996). A physical association between 52 kDa and 60 kDa Ro/hY RNA has been demonstrated by immunoprecipitation assays (Slobbe *et al*, 1991, 1992). Binding of 52 kDa Ro to hY RNA required the presence of 60 kDa Ro, strongly suggesting that 52 and 60 kDa Ro associate through protein-protein

interaction(s). As is the case for 60 kDa Ro, the function(s) of 52 kDa Ro are unknown, though sequence similarities and DNA-binding capability suggest it may act as a transcription factor (Itoh *et al*, 1991).

The La antigen is a 48 kDa protein that functions in RNA polymerase III transcription termination (Gottlieb and Steitz, 1989). Though La binds the 3' termini of numerous RNA polymerase III transcripts through a common oligouridylylate sequence, it is apparently stably associated with 60 kDa Ro through mutual binding to hY RNA (Hendrick *et al*, 1981). Additional sequence elements in a subset of hY RNA are also bound by La, which might account for the relatively stable interaction of La with 60 kDa Ro (Prujijn *et al*, 1991).

Calreticulin is a 46 kDa protein that was initially described as a calcium-binding protein of the endoplasmic reticulum, but its distribution within the cell is widespread, and its putative functions are numerous (Sontheimer *et al*, 1995). Calreticulin has been reported to bind both hY RNA and 52 kDa Ro (Cheng *et al*, 1996). Thus, it could form a molecular bridge linking the major Ro-associated molecules.

The physical association of macromolecular complexes of Ro-associated molecules may explain why antibodies to those molecules occur so frequently in the same sera. Over the past few years, several investigators have documented the phenomenon of epitope spreading (Bockenstedt *et al*, 1995; James *et al*, 1995; Topfer *et al*, 1995). Animals immunized to a peptide of an autoantigen can, with time, develop antibodies to other epitopes in the autoantigen, even though the epitopes are apparently disparate in shape. In some animals, antibodies may develop not only to epitopes of the original immunizing antigen but also to proteins with which the original antigen is physically associated. Epitope spreading has been reported in the cases of immunization with peptides of 60 kDa Ro, 52 kDa Ro, and La, and spreading of the anti-Ro response to calreticulin has been observed (Keech *et al*, 1996; Kinoshita *et al*, 1998; McCluskey *et al*, 1998). These observations of epitope spreading did not depend upon cross-reactivities among the proteins involved, but are rather thought to rely on B cell antigen presentation of multiple epitopes from various polypeptides within the Ro ribonucleoprotein complex (reviewed in McCluskey *et al*, 1998).

Although there are some reasonably well-developed hypotheses about how it is that multiple autoantibody specificities develop, it is still not clear how the original autoantibody response arises. One potential explanation is that an infectious agent containing an epitope cross-reactive with an epitope of a lupus autoantigen initiates the response. Once an epitope of the lupus autoantigen is recognized as foreign, epitope spreading may then occur. The recent population study of childhood lupus by James *et al*, showing a far greater prevalence of immunity to Epstein-Barr virus in the lupus population than in the control population, provides important evidence for this possibility (James *et al*, 1997). She further demonstrated a similarity between an Epstein-Barr virus epitope and an epitope of the lupus autoantigen, Sm.

Another possibility that is being explored is that the immune response begins in the skin, as a result of UV-induced damage to keratinocytes. Casciola-Rosen *et al* have reported that, during the process of UV-induced apoptosis, lupus autoantigens such as Ro and La are clustered within apoptotic cell surface blebs (Casciola-Rosen *et al*, 1994; Casciola-Rosen and Rosen, 1997). The blebs could theoretically provide a means for presenting Ro or La to the immune system in a context such that the autoantigens are recognized as foreign.

In summary, patients who have antibodies to Ro frequently have autoantibodies to more than one protein. The multiplicity of autoantibodies found in anti-Ro-positive sera may be explained by physical association of the autoantigens or by cross-reactivities. Some of the remaining uncertainties about the functions of the Ro-associated autoantigens, the clinical significance of antibodies to these antigens, and the role of specific autoantibodies in pathologic conditions, stem from the necessity of using patient sera, often containing multiple autoantibody specificities that are not necessarily trivial to detect.

THE CLINICAL SIGNIFICANCE OF ANTI-RO

Of the Ro-associated autoantibodies mentioned above, antibodies to 60 kDa Ro and La are the specificities about which there is the most

information. This is due in part to the fact that these specificities were the first to be discovered and in part to the fact that testing for anti-60 kDa Ro and anti-La is performed in commercial laboratories, often using relatively straightforward immunodiffusion assays, whereas testing for anti-52 kDa Ro and anti-calreticulin is generally limited to research laboratories.

If one examines all patients who have anti-Ro by immunodiffusion assays, there are numerous clinical findings that may occur. In an examination by Simmons-O'Brien of 100 patients who had anti-Ro, the following findings were present in 10% or more of the group (findings listed in order of frequency, with the most common first): arthritis, photosensitivity, Raynaud's phenomenon, nervous system disease, malar dermatitis, nephritis, pulmonary disease, alopecia, leukopenia, pericarditis, cutaneous vasculitis, DLE, decreased complement, SCLE, and anemia (Simmons-O'Brien *et al*, 1995). Clearly, only a minority of patients with antibodies to Ro develop SCLE.

If one examines all patients who have SCLE, however, the majority have anti-Ro. Admittedly, there is some controversy about the frequency of anti-Ro in SCLE, probably due to differences of opinion about what constitutes SCLE, as opposed to DLE, lesions. (For references concerning the distinction between SCLE and DLE, see David-Bajar *et al*, 1992; Sontheimer and Provost, 1996.) Notably, the group that originally defined SCLE identifies anti-Ro in the majority of patients (Sontheimer, 1989). Our group examined autoantibody specificities in 17 SCLE patients in detail, examining anti-60 kDa Ro, anti-52 kDa Ro, and anti-La quantitatively as well as qualitatively (Lee *et al*, 1994b). In our study, all SCLE patients examined had high titers of antibodies to 60 kDa Ro, and most had anti-52 kDa Ro as well. A minority concurrently had anti-La. These are approximately the same frequencies we found in neonatal lupus maternal sera (Lee *et al*, 1994a), supporting the relationship between NLE and SCLE and also illustrating that anti-60 kDa Ro is likely the major autoantibody specificity in these diseases.

The potential importance of anti-Ro in SCLE is underscored by the finding of anti-Ro in the skin. In immunosuppressed mice with human skin grafts, injection of anti-Ro antibodies led to preferential deposition of anti-Ro in the human skin in a particulate epidermal pattern identical to that seen in adult patients with SCLE and babies with NLE (Lee *et al*, 1986b). Our results were confirmed using purified anti-Ro antibodies and serum from which the anti-Ro specificity had been removed (Lee *et al*, 1989). These studies were performed prior to the discovery of 52 kDa Ro and the development of assays for anti-52 kDa Ro, so we do not know whether anti-52 kDa Ro contributed to the cutaneous deposits. Because epidermal IgG deposits were seen in the skin of an SCLE patient who had no detectable anti-52 kDa Ro by a sensitive ELISA test, it is likely that anti-60 kDa Ro is responsible for at least some of the antibody deposits observed, and also that anti-52 kDa Ro is not required for lesion formation.

The human skin-grafted mouse did not prove to be a good model for induction of SCLE lesions, and studies of SCLE have been hampered by the lack of such a model; however, models of cardiac disease in NLE have been developed, and these strongly support the possibility that anti-Ro antibodies cause disease (Miranda-Carus *et al*, 1998). Given the evidence available, it is reasonable to conclude that anti-Ro antibodies are necessary but not sufficient for disease to occur, both in adults with SCLE and in babies with NLE. This conclusion is somewhat simplistic, in that it is possible that other autoantibodies may on occasion substitute for anti-Ro. Nevertheless, the general principle applies that autoantibodies are likely to be necessary, but other factors must be present as well. This leads to a discussion of the role of UVR in the induction of lesions.

ULTRAVIOLET RADIATION AND THE INDUCTION OF SCLE

From a clinician's perspective, it is obvious that UVR contributes to the development of SCLE lesions. From the basic scientist's perspective, the mechanism of the association of UVR with SCLE is still to be fully explained (Table II). An early study by LeFeber *et al* indicated that UVR of cultured keratinocytes led to increased IgG binding to

Table II. Possible effects of UVR on cutaneous lupus

Modulation of autoantigen location
Apoptosis induction with autoantigens in apoptotic blebs
Induction of nitric oxide synthase expression
Upregulation of adhesion molecules
Expression of pro-inflammatory cytokines

the keratinocyte cell surface (LeFeber *et al*, 1984). Norris later noted increased antibody binding as a result of *in vivo* UV irradiation of human skin (Norris, 1993). Golan *et al* independently observed UV-induced binding of antibodies from anti-Ro-positive sera to a small percentage of cultured keratinocytes (Golan *et al*, 1992). One interpretation of these results is that UVR caused a translocation of Ro antigen to the cell surface; however, these studies did not employ purified antibodies, so the contributions of the various Ro-associated antibodies could not be assessed. In addition, antibody binding to the keratinocyte was the biologic read-out, and the putative cell surface antigen was not identified with molecular techniques. Thus, it is not clear whether the results can be explained by UV translocation of Ro (or other Ro-associated antigens) to the surface, induction of a cross-reactive antigen on the surface, or induction of apoptosis and movement of Ro into apoptotic blebs. Bachmann *et al*, using a monoclonal anti-La antibody, suggested that UV translocates La to the surface in the absence of apoptosis (Bachmann *et al*, 1990). Sontheimer *et al* reported that calreticulin appears on the cell surface as a result of UVR (Kawashima *et al*, 1994). However, the concept that intracellular antigens are translocated to the cell surface following UVR has been somewhat controversial because there is not an intuitive explanation for why it is that translocation would occur, at least in cells that are not committed to an apoptotic death pathway.

We have examined biopsies from patients with SCLE, examining sun-exposed lesional skin, sun-exposed normal-appearing skin, and unexposed normal-appearing skin (David-Bajar *et al*, 1992). All contained immunoglobulin deposits, and the sites sampled were indistinguishable with regard to pattern and intensity of immunoglobulin deposits. Thus, there is not a dramatic *in vivo* correlation to the experimental studies mentioned above, and differences in antibody binding induced by UVR in patients with SCLE would likely be small. One might postulate that in the baseline situation *in vivo* in patients with SCLE, antibody deposits are primarily intracellular. (The concept that antibodies may bind to the cell surface and then be taken up into a living cell is itself controversial, but there is experimental evidence to support this possibility (Koren *et al*, 1995; Koscec *et al*, 1997).) Following UVR, a transient increase in antibody binding to the surface may occur, making the keratinocyte more susceptible to killing by complement or antibody-dependent cellular cytotoxicity. This hypothesis could reconcile the previous observations of increased antibody binding following UVR with the failure to detect increased antibody deposits *in vivo* in the sun-exposed skin of SCLE patients; however, there is not evidence to prove or disprove this hypothesis.

Rather than, or in addition to, acting upon antibody binding, UVR may exert its effects at points distal to antibody deposition in keratinocytes. Sontheimer *et al* have reported that animals pre-treated with anti-Ro have a lower threshold for UV-induced erythema (Davis *et al*, 1989). The mechanism of this effect was not explained, and it remains to be determined whether and how anti-Ro affects susceptibility to UV-induced apoptosis, erythema, and inflammation.

Kuhn *et al* reported that patients with cutaneous LE have aberrant cytokine-induced nitric oxide synthase (iNOS) regulation following UVR. Compared with normal controls, LE patients exhibited delayed onset but considerable prolongation of iNOS expression (Kuhn *et al*, 1998). How and if this interesting phenomenon is affected by autoantibodies is not yet known.

The effects of UVR on adhesion molecule upregulation and pro-inflammatory cytokine expression are likely to be relevant in affecting lymphocyte migration into the skin. For example, UVR induces a delayed upregulation of ICAM-1 expression in exposed human keratinocytes, which is thought to be mediated by pro-inflammatory cytokines (Norris, 1990; Krutmann and Grewe, 1995; Middleton and

Norris, 1995). Notably, particular patterns of ICAM-1 expression have been documented in various types of interface dermatitis, with diffuse epidermal expression occurring in case of SCLE (Bennion *et al*, 1995). Lymphocytes that traffic to the skin could conceivably interact with the ICAM-1 expressing keratinocytes to produce keratinocyte damage. Alternatively, lymphocytes in the skin could act mainly by releasing cytokines. A recent report that transgenic mice overexpressing gamma interferon in the epidermis develop anti-dsDNA and anti-histone autoantibodies gives credence to the possibility that cytokines released from lymphocytes could contribute to lupus (Seery *et al*, 1997). The relevance of this interesting finding to cutaneous lupus is not yet known, however. In any case, because a lymphocytic infiltrate is a hallmark of the pathology of active SCLE lesions, lymphocytes are probably critical to lesion formation, but the contribution of lymphocytes to the pathology of the skin lesions has not been established.

THE LESSONS OF NLE

The reproduction of disease in infants whose mothers have anti-Ro has proved to be invaluable in understanding the mechanisms whereby SCLE lesions might occur (Lee, 1993). Some of the more important clinical observations are as follows: the cutaneous lesions of NLE are histologically and by immunofluorescence indistinguishable from SCLE in adults. Like SCLE in adults, the lesions do not typically scar. Almost without exception, children with cutaneous NLE lesions have maternal anti-Ro. The cutaneous disease may be present at birth, but more often appears shortly after birth. In some cases, lesions occur following sun exposure; however, lesions may appear in relatively sun-protected areas such as the nappy area. The duration of disease activity ranges from weeks to months. By 1 y of age at the most, the disease has resolved. Although virtually all babies with NLE have anti-Ro, only a small fraction of babies whose mothers have anti-Ro develop NLE. Even in cases of twins, it is not unusual for one twin to be affected and the other to be unaffected. There are four major clinical manifestations of NLE, but it is unusual for a given baby to have more than one or two. These are cutaneous lupus, congenital heart block with or without cardiomyopathy, cholestatic liver disease, and thrombocytopenia.

Considerable laboratory investigation has been done in NLE and some of the more significant findings are as follows: Conduction abnormalities, including heart block, can be reproduced in animal models by anti-Ro (Alexander *et al*, 1992; Garcia *et al*, 1994; Boutjdir *et al*, 1998; Miranda-Carus *et al*, 1998). It is likely, given the information available, that either anti-52 kDa or anti-60 kDa Ro can produce heart block. Whereas in our studies anti-60 kDa Ro was found in all NLE sera tested, anti-52 kDa Ro is also exceedingly common, and both autoantibodies are candidates for pathogenic autoantibodies in this disease. The factors responsible for the targeting of one organ *versus* another are not known. Antibody deposits can be found in all organs examined, including organs that are never apparently affected in NLE (Reichlin *et al*, 1994). Autoantibody profiles are not demonstrably different in mothers who have babies with heart block compared with mothers who have babies with skin disease. In our group, however, the mothers who had babies with skin disease had lower titers of circulating anti-60 kDa Ro than did mothers who had babies with heart block; this is a possible indication of differences in autoantibody–autoantigen interactions, but the significance of this observation is not yet known (Lee *et al*, 1994a).

From these data, one may conclude that autoantibodies are necessary for disease to occur, but are probably insufficient. The appearance of disease in one twin but not the other indicates that autoantibody specificities alone do not determine whether an individual will be affected or which organs will be involved. In addition, the finding in autopsy studies and in animal models of antibody deposits in all organs examined indicates that antibody deposition alone does not determine disease expression (Reichlin *et al*, 1994).¹ It is unclear what other

factors come into play to produce disease, whether genetic factors, environmental factors, or a set of random events. UVR is likely an exacerbating factor in skin disease, but the appearance of lesions at birth in some babies, and the occasional appearance of lesions in the nappy area likely means that UVR is unlikely to be required for cutaneous disease. Interestingly, although a lymphocytic infiltrate is a hallmark of active cutaneous disease, inflammatory infiltrates are often not detected in autopsy studies of affected hearts and the significance of lymphocytes in the pathogenesis of NLE and SCLE is uncertain.

LINKING ANTI-RO WITH DLE

The earlier mentioned studies of SCLE and NLE provide reasonably convincing evidence for a link between anti-Ro or a closely related autoantibody specificity and photosensitive cutaneous lupus. Is there a role for anti-Ro in the more common form of cutaneous lupus, DLE? Using a standard immunodiffusion (Ouchterlony) technique, the majority of patients with DLE do not have anti-Ro, and in our studies of antibody deposition, patients with DLE do not have the epidermal IgG deposits that can be attributed to anti-Ro but rather have antibody deposits at the dermal–epidermal junction (Lee *et al*, 1994b). Furthermore, a substantial number of DLE patients are not photosensitive. This would seem to indicate that anti-Ro is associated specifically with photosensitive cutaneous lupus, and in particular, SCLE.

In light of the above, it was surprising that, in a study of autoantibody specificities in SCLE and DLE, we found a majority of DLE patients to have anti-Ro (Lee *et al*, 1994b). Eleven of 15 DLE patients had IgG anti-Ro by sensitive ELISA techniques. This was largely anti-60 kDa Ro, although a few had anti-52 kDa Ro. There was not a clear association between the presence of anti-Ro and the clinical finding of photosensitivity in the DLE patients. Consistent with previous studies, a very low frequency of anti-Ro was present in the DLE patients if standard immunodiffusion was performed; only when the much more sensitive ELISA techniques were used were the autoantibodies detected. The likelihood that the ELISA data were genuine was confirmed by blocking the reactivity of the sera with purified antigen. In addition, dermatomyositis patient control sera showed a significantly lower frequency of anti-Ro, so the results were unlikely to be a non-specific result of having autoimmune cutaneous disease. One possibility for the high frequency of low-titer anti-Ro in DLE sera is that the immune response to Ro is intimately linked to cutaneous lupus, and that in SCLE the response is primarily an autoantibody response, whereas in DLE the response is primarily cell-mediated. This possibility fits well with the observation that SCLE lesions but not DLE lesions occur in newborns, as IgG autoantibodies cross the placenta with ease, whereas lymphocytes apparently do not. Another possible interpretation of the autoantibody data is that DLE patients have an associated autoantibody specificity that is not anti-Ro but is weakly cross-reactive with Ro.

In summary, there is an intriguing possibility that anti-Ro or closely related autoantibodies are highly linked to cutaneous lupus, whether SCLE or DLE, but the true relationship between DLE and anti-Ro has yet to be elucidated.

PHOTOSENSITIVE CUTANEOUS LE UNASSOCIATED WITH ANTI-RO

To conclude that anti-Ro is unrelated to photosensitive cutaneous lupus is to overlook a great deal of evidence to the contrary; however, anti-Ro may not be the entire explanation for photosensitive cutaneous lupus. Particularly in photosensitive DLE, patients may not have detectable anti-Ro. One possibility, raised above, is that the immune response to Ro in certain patients may be primarily cell-mediated. Although that explanation is attractive in certain respects, particularly with regard to explaining why DLE lesions are rarely, if ever, seen in infants, it does not explain the reasonably consistent presence of antibody deposits at the dermal–epidermal junction in DLE lesions. One would particularly like to know the specificity (-ies) of the antibodies deposited at the dermal–epidermal junction, but this information is not currently available. Because the dermal–epidermal antibody deposits in DLE are often IgM, perhaps DLE is an IgM-mediated

¹Lee LA, Coulter S, Norris D, Weston WL: An animal model for studying transplacental passage and tissue deposition of antibody in neonatal lupus. *Clin Res* 34:161A, 1996 (abstr.)

Table III. Variants of cutaneous LE and their responses to UV

	Clinically photosensitive ^a	Lesions reproducible by phototesting ^b	Autoantibody associations	Depth of inflammatory infiltrate
ACLE ^c	very frequent	minority	anti-dsDNA	superficial
SCLE	very frequent	majority	anti-Ro and related abs	superficial
DLE	occasional	minority	possibly, low titer anti-Ro	superficial and deep
DDLE	often	minority, but more than DLE	?	superficial and deep
TLE	often	majority	?	superficial and deep
LEP	rare	?	?	deep

^a"Clinically photosensitive" indicates that the patient gives a history of photosensitivity. The estimates listed represent the anecdotal experience of the one of the authors (LAL). Not all investigators agree, however, with the high frequency of photosensitivity in ACLE and SCLE.

^bData extracted and summarized from Lehmann *et al* (1990), Walchner *et al* (1997), and Wolska *et al* (1989).

^cACLE, acute cutaneous LE; SCLE, subacute cutaneous LE; DLE, discoid LE; DDLE, disseminated discoid LE; TLE, tumid LE; LEP, LE panniculitis.

disease that does not occur in infants because IgM does not cross the placenta. In any case, searches by our laboratory for specific IgM autoantibodies in DLE sera have not been fruitful. Finally, photosensitive cutaneous lupus in patients who do not have anti-Ro could be related to other IgG antibody specificities, such as anti-U1RNP, which has been found in a handful of cutaneous NLE patients (Provost *et al*, 1987).

PHOTOTESTING

A major impediment to the understanding of the pathogenesis of photosensitive cutaneous lupus is the lack of an optimal animal model of disease. For example, although the cardiac conduction defect of NLE has been reproduced, the cutaneous SCLE lesions have not. Reproduction of disease in humans would be extremely useful, but has been difficult to achieve. Although reproduction of lesions in patients with known cutaneous lupus does not allow for the study of events that take place long before the first cutaneous lesion becomes evident, it would at least illuminate the events that take place proximate to lesion formation. Recently, protocols for phototesting have been optimized by taking into account multiple parameters such as light source, area of skin irradiated, site of irradiation, dose of UV, frequency of irradiation, and time to induction of lesions. All of these factor into success or lack of success of phototesting (Walchner *et al*, 1997).

Phototesting has confirmed that some patients with cutaneous lupus have lesions that can be initiated by UV exposure, and has substantiated differences in clinical subsets of cutaneous lupus with regard to response to UV (Table III). In general, lesions of SLE (presumably, acute cutaneous lupus) can be reproduced in about one-fourth of patients tested, lesions of SCLE in about two-thirds, and lesions of DLE in about one-third (Wolska *et al*, 1989; Lehmann *et al*, 1990; Walchner *et al*, 1997). Within the DLE group, patients who have disseminated lesions are much more likely to have lesions reproduced by phototesting. Wolska *et al* were able to reproduce lesions in nine of 21 patients with disseminated DLE but only in nine of 90 patients with limited DLE (Wolska *et al*, 1989). A somewhat surprising finding is the high frequency of photo-reproduction of lesions in patients who have tumid lupus. Tumid lupus is an unusual variant characterized by indurated erythematous plaques that appear morphologically similar to edematous plaques of polymorphous light eruption. About four-fifths of patients with tumid lupus had lesions reproduced in phototesting (Lehmann *et al*, 1990; Walchner *et al*, 1997). Further studies using photo-reproduction of lesions should result in better understanding of the events that occur during lesion formation.

THE FUTURE

As should be evident from this review, it is clear that UV radiation plays a major role in the induction of lesions in some patients with cutaneous lupus, particularly patients with SCLE; however, the mechanisms whereby UV radiation results in lesion formation have not been fully elucidated. In the future, studies of pathomechanisms of lesion formation using phototesting of humans with lupus should help identify the steps involved in the induction of lesions. In addition, studies of the complex genetics of lupus have the potential to identify factors involved in disease formation, including factors involved long before disease becomes evident. Finally, determination of the functions of the lupus autoantigens, such as Ro, along with the effects of autoantibody binding on autoantigen function or other cellular functions may help explain the relationship of specific autoantibodies to cutaneous disease.

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