IgG Subclasses in the Serum and Skin in Subacute Cutaneous Lupus Erythematosus and Neonatal Lupus Erythematosus

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IgG subclasses differ in their biologic and chemical properties, such as complement fixation, protein and cellular binding, and placent transfer. In this study, IgG subclasses of anti-Ro/SSA antibodies in subacute cutaneous lupus (SCLE) and neonatal lupus (NLE) are examined in the serum and in the skin. IgG subclasses in NLE beginning in utero (NLE-heart disease) are compared to subclasses in NLE beginning after birth (NLE-skin disease). Human skin was grafted onto athymic mice, mice were injected with one of eight anti-Ro/SSA maternal NLE sera (four heart block, four skin disease) or seven anti-Ro/SSA SCLE sera, and grafts were examined for IgG subclasses using monoclonal anti-human IgG subclass reagents in an immunofluorescent technique. Lesional skin was examined from four SCLE patients. IgG1 was the only IgG subclass detected in the grafts and skin lesions. IgG1 was the predominant anti-Ro/SSA IgG subclass detected in SCLE and NLE sera in an ELISA using a synthetic Ro/SSA polypeptide. These studies show that the maternal anti-Ro/SSA autoantibodies in NLE-heart disease sera are predominantly IgG1 and are therefore likely to be present in the fetus at the time of gestation, when heart block usually develops. Second, differences in the clinical presentations of NLE (in utero vs. postnatal disease) cannot be attributed to differences in anti-Ro/SSA IgG subclasses. Finally, the subclass bound in the skin in SCLE is IgG1, a subclass capable of mediating tissue injury via complement or cellular effectors. J Invest Dermatol 95:643–646, 1990

One of the characteristic clinical findings in subacute cutaneous lupus erythematosus (SCLE) and neonatal lupus erythematosus (NLE) is a photosensitive skin eruption that typically occurs in association with autoantibodies, particularly anti-Ro/SSA [1,2]. The course of the subacute cutaneous skin lesions in infants with NLE is linked to the presence of maternal anti-Ro/SSA antibody, which presumably crossed the placenta prenatally [3].

Lee et al have conducted animal studies to elucidate the relationship between the anti-Ro/SSA antibodies found in SCLE and NLE sera and the skin lesions found in these patients. Using an experimental model, the nude mouse with grafted human skin, they described epidermal binding of IgG anti-Ro/SSA antibodies in a particular pattern. This pattern of binding was seen both in the human skin grafts and in the lesions of SCLE and NLE patients [4,5]. These studies, demonstrating anti-Ro/SSA in the skin, together with clinical observations that skin lesions in newborns resolve as circulating maternal anti-Ro/SSA antibodies are no longer detectable, provide evidence for the hypothesis that anti-Ro/SSA antibodies are involved in the pathogenesis of NLE and SCLE.

Infants with NLE generally have either skin disease, which usually begins after birth, or complete congenital heart block (CHB), which begins in utero. The CHB may be detected as early as the 16th week of gestation [6]. Differences in placental transmission of the IgG subclasses have been observed in several studies [7–9]. IgG1 is consistently found to cross the placenta relatively well [7–9], whereas IgG2 has been reported in one study to have significantly impaired placental transfer [8]. It is not known if the anti-Ro/SSA autoantibodies in NLE with CHB are of the IgG1 subclass and therefore are likely to be present in the fetal circulation at the gestational age when heart block may develop. It is also not known what the IgG subclass composition of anti-Ro/SSA is in NLE without heart block (e.g., NLE-skin disease). A difference in IgG subclasses could conceivably have bearing on the different clinical presentations of NLE, i.e., the in utero damage of CHB versus the postnatal skin lesions, if different subclasses of anti-Ro/SSA IgG are represented in CHB patients rather than in skin disease patients.

Lee et al have studied the sera of adult SCLE patients and discovered that circulating anti-Ro/SSA reactive with a major Ro/SSA epitope is composed largely of the IgG1 subclass [10]. Because IgG1 readily fixes complement [7] and mediates antibody-dependent cel-
lular cytotoxicity (ADCC) [10], it has been hypothesized that it may play a role in the production of SCLE skin lesions. However, antibody deposition in the skin was not examined.

The purposes of this study are to determine the IgG subclass composition of anti-Ro(SSA) antibodies in NLE and SCLE, to determine whether the IgG subclass composition of the maternal sera of NLE patients with immune damage (CHB) is different from that of maternal sera of NLE patients with postnatal disease (skin lesions), and to determine the subclasses bound to the skin in SCLE.

Our approach was to examine IgG subclass binding in vivo to native antigen(s) in the grafted human skin of the nude mouse and in SCLE patients’ skin lesions. Grafted mice were injected with maternal NLE serum or SCLE serum, and the grafts examined for IgG subclass deposition. Lesions of SCLE patients were examined for IgG subclass deposition. As a confirmatory measure, circulating anti-Ro(SSA) subclasses were also examined with an enzyme-linked immunosorbent assay (ELISA) using an antigenic Ro(SSA) polypeptide.

**MATERIALS AND METHODS**

**Human Skin Graft Procedure** Human skin was obtained from cosmetic procedures involving the face, abdomen, and breast. This tissue would otherwise have been discarded and was obtained in accordance with the policies of the Institutional Review Board for Human Investigation. Using a Davol dermatorome, split-thickness skin grafts approximately 400 μm in thickness were harvested and grafted onto Hsd:athymic nude-nu AF mice using the method of Krueger and Briggaman [11]. After approximately 3–4 weeks, the grafts were healed and the mice were injected with SCLE, NLE, or normal sera.

**Antisera and Patient Characterization** The antibody composition of the various sera used for injection of the mice was determined by using the Ouchterlony double-diffusion test described by Nakamura et al [12] and by using a western blotting technique previously described [13]. Using this method, it was determined that all SCLE patients’ sera contained anti-Ro(SSA) without other autoantibodies being present. Of the eight sera from NLE mothers, three contained anti-Ro(SSA) and five contained both anti-Ro(SSA) and anti-La(SSB) [14]. The sera from two normal patients was utilized for negative controls.

All SCLE patients had signs and symptoms consistent with SCLE and histology consistent with cutaneous lupus. Of the eight infants with NLE, four had heart block without skin lesions and four had skin lesions without heart block.

**Injection and Immunofluorescent Techniques** After the human skin grafts had healed, the mice were injected intra-peritoneally with 0.1 cc of sera from one of seven SCLE patients, one of eight mothers of NLE babies, or the two normal controls. The grafts were harvested after 24 h and sectioned. To confirm that the area contained human skin, sections of the graft were examined with the Hoescht stain (Flow Laboratories, McLean, VA, dilution 1:5), which can distinguish human from mouse nuclei [15].

The subclass epidermal staining incorporated a double-staining technique using a primary anti-human IgG subclass mouse monoclonal antibody with a 2-h incubation, and a secondary stain of goat anti-mouse IgG fluorescein-conjugated antibody for 2 h (Tago Inc, Burlingame, CA, dilution 1:100).

**Monoclonal Anti-Human IgG Subclass Reagents** The monoclonal antibodies used in this study were anti-IgG1 HP6012, anti-IgG2 HP6014, anti-IgG3 HP6047, and anti-IgG4 HP6025 (World Health Organization Collaborating Center for Research and Reference Reagents for Human Immunoglobulins at the Centers for Disease Control). Equivalent dilutions of the monoclonal IgG subclass reagents for immunofluorescence tissue staining were determined using an ELISA and measuring the optical density (OD) values after a reaction between each subclass reagent and its respective purified myeloma IgG subclass protein, as described in a previous study [16]. The OD values were roughly equivalent among the subclass reagents and a dilution of 1:100 was made.

**Human Tissue Biopsies** Four patients with SCLE skin lesions and anti-Ro(SSA) positive sera were biopsied in a lesional site. The tissue was snap-frozen, and sections were stained with subclass reagents as noted above.

For positive controls, lesional skin was obtained from two patients with biopsy and IF-proved bullous pemphigoid. The tissue was snap-frozen in liquid nitrogen sectioned and stained with subclass reagents as noted above.

**Anti-Ro(SSA) IgG Subclass Enzyme-Linked Immunosorbent Assay (ELISA)** Synthetic peptide (7–24) of human Wil-2 Ro(SSA) was coated on 0.5% glutaraldehyde-treated Immunulon II microtiter plates at 4°C for 16 h. This peptide has been determined by Liu et al [10] to be one of the major epitopes recognized by anti-Ro(SSA) sera. The remaining sites in the wells were blocked by adding 0.2 ml of 1% bovine serum albumin (BSA) dissolved in phosphate-buffered saline (PBS)-Tween. Sera to be tested for antibody IgG subclasses were diluted (1:100) with serum diluent (PBS-Tween containing 1% BSA and 0.5% bovine gamma globulin) added to the plates and incubated at room temperature for 2 h. After washing, mouse monoclonal anti-human IgG subclass antibodies were added and the plates were incubated for 2 h. Biotinylated goat anti-mouse IgG (Tago, Inc, Burlingame, CA, dilution 1:1000) was added for 2 h. Then, peroxidase-conjugated avidin (1:1000) was added for 2 h. The color was developed with 1 mg/ml of 2,2-azino-di-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 0.005% hydrogen peroxide in 0.1 M Melvaine’s buffer, pH 4.6. The OD was measured by a Titertek Multiskan Reader. Samples were run in triplicate. Data were analyzed with a Student’s t test, and values greater than 3 standard deviations (SD) above the mean for normal sera were considered significant.

**RESULTS**

Fifty-five nude mice were grafted and injected with sera from seven SCLE patients and sera from the mothers of eight NLE patients (four with heart block only and four with skin lesions only). IgG1 staining was consistently positive in all samples taken from human skin grafts. Staining with the other IgG subclasses was not seen in any of the grafts except for occasional, presumably non-specific, staining of Civatte bodies with IgG2 and IgG3.

The immunofluorescence staining pattern shown in Fig 1 is typical of the findings in all human graft biopsies. The location of bound IgG1 is epidermal and in a particulate nuclear and cytoplasmic pattern.

Controls consisting of human graft biopsies from mice injected with normal human sera were invariably negative when stained for all IgG subclasses. This was an expected result because, in previous work, none of 40 mice injected with eight different normal sera had IgG deposition in their skin grafts [4; Lee LA, unpublished data]. Skin biopsies of perilesional skin from bullous pemphigoid patients were used as positive controls to establish that the IgG 2, 3, and 4 subclass antibodies were functional in the immunofluorescence assay. Positive staining was demonstrated for IgG 2, 3, and 4 in these controls.

The lesions from four SCLE patients were biopsied and stained for IgG subclasses. The findings were similar to those in the nude mouse model. The epidermal staining pattern for IgG1 was particulate nuclear and cytoplasmic staining.

The ELISA results for the circulating anti-Ro(SSA) antibodies binding to an antigenic Ro(SSA) polypeptide are presented in Fig 2. IgG1 was the predominant anti-Ro(SSA) subclass represented. There were elevations of IgG1 anti-Ro(SSA) antibodies in six of the eight NLE patients and three of the seven SCLE patients. Minimally
Figure 1. Immunofluorescent staining for IgG1 in the human skin graft in a mouse injected with anti-Ro/SSA sera reveals epidermal staining in a granular pattern in the epidermis with accentuation in and just above the basal keratinocytes. (E, epidermis; D, dermis.)

Elevated IgG2 was seen in one NLE serum, and IgG4 in two NLE and one SCLE sera. IgG3 anti-Ro/SSA was detected in three NLE sera (two heart block and one skin disease sera) and five SCLE sera. The magnitude of the elevations with IgG3 was significant, but less than with the IgG1 subclass.

DISCUSSION

Although NLE and adult SCLE are clinically distinct, the similarity of skin lesions and the usual presence of anti-Ro/SSA autoantibody in the sera of both suggests they are linked. Speculation that these autoantibodies play a role in the pathogenesis of these diseases has been fueled by a series of recent findings.

The initial evidence that anti-Ro/SSA is important in the pathogenesis of skin lesions in NLE was presented by Weston et al in their study of NLE [3]. Their study clearly demonstrated an association between the presence of skin lesions and circulating maternal anti-Ro/SSA antibodies in NLE infants.

The development of the nude mouse model for antibody binding in SCLE led to the discovery that anti-Ro/SSA was bound to grafted human skin in mice injected with affinity-purified anti-Ro/SSA antibodies [4,5]. The binding was found principally in the epidermis in a particulate pattern. This staining was blocked by prior adsorption of anti-Ro/SSA serum to purified Ro/SSA antigen, confirming that this epidermal staining was in fact due to binding by anti-Ro/SSA [5]. Further, it was shown that the epidermal staining was not produced artifactualy during sectioning of skin, as had previously been hypothesized [5].

Thus, anti-Ro/SSA antibodies are consistently found in sera of patients with SCLE and NLE, have a temporal association with skin disease in NLE, and have been shown to bind to human skin.

The subclasses represented in anti-Ro/SSA IgG are of interest because the four IgG subclasses may be functionally different and may cross the placenta differently. Subclasses differ in their ability to fix complement, to bind proteins such as protein A and rheumatoid factor, and to bind cells such as macrophages and lymphocytes [7]. IgG1, for example, is an efficient activator of complement and mediator of ADCC [10].

A study of placental transmissibility of antibodies concluded that transfer of IgG subclasses across the placenta is an active process, with IgG2 transfer being especially impaired [8]. IgG subclasses have been observed to have different binding affinities to placental tissue, with IgG1 having the greatest and IgG2 having the least affinity [17]. On the other hand, one study found no major impairment of transfer of any subclass [9]. However, in that study there were differences observed in placental transfer among the subclasses, with IgG1 reaching maternal levels at an earlier time than did the other subclasses.

Our studies have revealed that IgG1 is the only IgG subclass detectable in human skin grafts in mice injected with SCLE and NLE sera, the only IgG subclass found in SCLE lesional skin, and the predominant subclass reactive with a major Ro/SSA epitope in an ELISA.

IgG1 is likely to be present in the fetal circulation at a time during gestation when heart block due to NLE may develop. Thus, the finding of IgG1 anti-Ro/SSA in NLE-heart block sera provides further support to the hypothesis that the anti-Ro/SSA autoantibodies cross the placenta and are then directly involved in the tissue injury seen in NLE.

Most infants with NLE have either heart disease or skin disease. Heart disease, characteristically, complete congenital heart block, begins in utero and has been detected as early as the 16th week of gestation. Skin disease usually begins shortly after birth. In our study, both groups had IgG1 anti-Ro/SSA. Thus, despite exposure to an IgG subclass that crosses that placenta at a time when conduc- tion system disease may develop, some babies exposed to IgG1 antir Ro/SSA do not develop heart block. The explanation for differ- ences in the clinical expression of NLE must lie somewhere other than in differences in anti-Ro/SSA IgG subclasses.

Figure 2. This scattergram depicts ELISA results for reactivity of anti-Ro/SSA IgG subclass antibodies with the N-terminal (residues 7–24) Ro/SSA polypeptide. Values for anti-Ro/SSA IgG1, 2, 3, and 4 antibodies are listed as standard deviations (SD) from normal values for each subclass. Dotted line, mean; dashed line, upper limit of normal. NLE sera are denoted by open circles and SCLE sera by closed circles.
Previous work has demonstrated that IgG1 is the predominant subclass of anti-Ro/SSA reactive with a major Ro/SSA epitope in an ELISA [10]. We have shown that IgG1 is the only subclass of IgG detectable in an in vivo antibody binding assay using SCLE and NLE sera. The absence of IgG3 staining in the grafts and skin lesions is interesting in light of the finding of IgG3 anti-Ro/SSA in some sera in the ELISA assay. Although it is possible that the IgG1 anti-Ro/SSA antibodies are selectively deposited in the skin, it is perhaps more likely that the findings are indicative of a greater circulating level of anti-Ro/SSA IgG1 than IgG3. Conversely, the ELISA assay did not detect anti-Ro/SSA IgG1 in some sera in which IgG1 binding to tissue was demonstrated. An explanation for this discrepancy may lie in the character of the Ro/SSA antigen used in the assay. Although the synthetic Ro/SSA antigen used in this study is a major Ro/SSA epitope, other areas of the same Ro/SSA molecule have also been demonstrated to be significant epitopes [18]. Furthermore, completely different Ro/SSA molecules have been isolated and characterized [19, 20]. Because the Ro/SSA antigen used in our assay represented only one of many possible Ro/SSA epitopes, the ELISA results may have missed some sera that contained anti-Ro/SSA antibodies targeted towards other Ro/SSA epitopes. However, the in vivo antibody binding assay using the human skin grafts should detect binding to all significant Ro/SSA epitopes, both to epitopes within one Ro/SSA protein and to epitopes on different Ro/SSA species present within the tissue.

Our findings in SCLE and NLE strongly militate against the importance of any IgG subclass besides IgG1 in the production of SCLE and NLE skin lesions. Further studies will be necessary to determine conclusively the role of anti-Ro/SSA autoantibodies in the pathogenesis of cutaneous lesions in SCLE. Nevertheless, these and previous studies have shown that anti-Ro/SSA autoantibodies are present in an appropriate location in SCLE and NLE to mediate skin disease, and that the subclass present is such that complement and/or antibody-dependent cellular cytotoxicity could be involved in the pathogenesis of skin injury.

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