Antibodies to double-stranded DNA in systemic lupus erythematosus. Parameter for disease activity and treatment

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Introduction

The serological hallmark in systemic lupus erythematosus is the presence of antibodies against DNA. The studies described in this thesis center around the induction of these antibodies, and the significance of anti-dsDNA in relation to diagnosis and disease activity in SLE. In addition, clinical measurements of disease activity and chronicity were evaluated in relation to the practical clinical use of anti-dsDNA as parameter of disease activity.

Induction of antibodies against DNA

Clinical and experimental data support a role for anti-dsDNA antibodies in the pathogenesis of SLE. The stimulus for the induction and further production of anti-dsDNA in SLE is, however, unknown. Various experimental data suggest a role for the microflora in auto-immune diseases. With respect to SLE, invasion of foreign bacteria from the intestines may result in polyclonal activation of the immune system and/or induce anti-dsDNA antibodies by a process of molecular mimicry. Indeed, bacterial DNA has been shown to be immunogenic. Normally, invasion of foreign bacteria is prevented by various host factors amongst which the colonization resistance is of primary importance. The colonization resistance is the defence capacity of the indigenous microflora against colonization by foreign bacteria. We found that SLE patients, both during inactive and active stages of the disease, tended to have a colonization resistance lower than healthy individuals *(Chapter 2)*. A lower colonization resistance could imply that in SLE patients more different bacteria may translocate across the gut wall. Some of these bacteria may serve as antigen for the production of anti-bacterial antibodies cross-reacting with autologous DNA. In *Chapter 3*, we found that in both inactive and active SLE patients IgM titres against their own faecal microflora were lower in comparison to healthy individuals. This was interpreted as a failing network against bacterial antigens in SLE. IgG-class antibacterial antibodies were increased in inactive patients and decreased in active patients. The former is consistent with a higher degree of bacterial translocation. We suggested that the observed decrease of IgG-class antibacterial antibodies during active disease might result from usage of these antibodies in formed immune-complexes. A similar phenomenon, i.e. a sharp decrease in anti-dsDNA has been shown during relapses of SLE. To analyze the specificity of the involved antibodies, with respect to their reactivity to bacterial antigens from the host

microflora, dissociation of immune complexes from sera from patients with active disease has to be performed yet.

Measurement of anti-dsDNA, diagnostic tool and target for treatment in SLE

For the determination of anti-dsDNA different assay methods are available, which differ in their sensitivity and specificity with respect to the diagnosis of SLE. In *Chapter 4* we determined the diagnostic value of three different assays (Farr, ELISA and Crithidia luciliae immunofluorescence test) and concluded that the Farr assay, using ^{125}I recombinant DNA as substrate, appeared to be the test with the highest specificity and sensitivity for SLE. In this cross-sectional study special attention was paid to the diagnostic significance of IgM-class anti-dsDNA. In SLE, but also in other auto-immune diseases IgM-class anti-dsDNA could be detected. We found that all SLE sera positive for IgM-class anti-dsDNA but negative for IgG-class anti-dsDNA as detected by ELISA, were positive by the Farr assay. In contrast, most of the sera positive for IgM-class anti-dsDNA from patients with diseases other than SLE were negative when tested by the Farr assay.

In *Chapter 5*, we investigated in a longitudinal study, the predictive value of rises in IgM-class anti-dsDNA by ELISA for ensuing relapses in SLE, in comparison to rises in IgG-class anti-dsDNA by ELISA and rises in anti-dsDNA as detected by Farr assay. We found that the cumulative risk for relapses did not differ significantly between patients with rises in IgM-class anti-dsDNA and those without a rise in IgM-class anti-dsDNA. Moreover, no specific clinical manifestations of SLE were associated with rises in IgM-class anti-dsDNA antibodies. In contrast, patients with rises in IgG-class anti-dsDNA measured by ELISA and anti-dsDNA by Farr assay had a significantly higher cumulative risk for relapses than patients without those rises.

Clinical measures of disease activity in systemic lupus erythematosus

In order to assess prognosis and to evaluate the effect of different treatment strategies in SLE patients it is evident that both clinical disease and damage measures are required. Three indices, the Systemic Lupus Activity Measure (SLAM), the SLE Disease Activity Index (SLEDAI) and the British Isles Lupus Assessment Group (BILAG), are considered to be reliable and validated measures of clinical disease activity in patients with SLE. In *Chapter 6* we compared these indices in their sensitivity to measure change over time. We

concluded that SLAM appeared to be most sensitive to measure change in disease activity over time. BILAG was slightly less sensitive compared to SLAM, but has the advantage of measuring disease activity per organ system. SLEDAI appeared to be the less sensitive. In addition, we demonstrated that changes in disease activity measured by SLAM and BILAG correlated significantly with changes in levels of anti-dsDNA. The overall use of one index would greatly enhance the possibility of comparing clinical studies from various centers.

Measurement of anti-dsDNA as guide for treatment in SLE

The observed risk for relapses after rises in IgG-class anti-dsDNA by ELISA and rises in anti-dsDNA determined by Farr assay *(Chapter 5)* were in accordance with a previous finding, showing that rises in IgG-class anti-dsDNA by ELISA preceded a clinical relapse in 74% of the cases, and rises detected by Farr assay in 89% of the cases. Rises in anti-dsDNA could already be detected 8 to 10 weeks before the relapse occurred. Accordingly, we performed a prospective randomized controlled trial aimed at the prevention of clinical relapses in SLE patients by treatment based on rises in levels of anti-dsDNA *(Chapter 7)*. When a rise in anti-dsDNA was detected, patients were randomly assigned either to conventional treatment or to early treatment with 30 mg prednisone in addition to current therapy. In the group of patients assigned to conventional treatment (n=24) 20 out of the 24 patients developed a relapse, whereas only two relapses occurred in the early treatment group (n=22). The mean daily doses of prednisone differed significantly between the two treatment arms, although the cumulative doses of prednisone did not differ the groups. Thus, changes in anti-dsDNA antibodies can be used as target for treatment. Further studies should reveal whether the dose of prednisone required for prevention of relapses can be reduced or whether specific therapy aimed at the eradication of anti-dsDNA producing B cells is possible.

Measuring of damage in systemic lupus erythematosus

In SLE patients, it is important to be able to evaluate the effect of treatment not only in terms of reducing disease activity, but also in terms of reducing accumulated damage over time. Recently, an index for damage in SLE patients, the SLICC/ACR (Systemic Lupus International Collaborative Clinics/

American College of Rheumatology) damage score has been developed. Damage in SLE may result from previous disease activity leading to organ failure, from medication, or from intercurrent illness, such as surgery or cancer. In *Chapter 8* we evaluated the usefulness of this damage score in SLE. This damage index appeared to be sensitive to measure change over time. The damage index reflects cumulative disease activity, especially of kidneys and haematologic systems, as well as cumulative doses of prednisone. We used this damage score in the clinical trial described in *(Chapter 7)* and found that in both treatment arms the damage score increased significantly, although the change in damage score was not significantly different between both treatment groups. We concluded that the SLICC/ACR damage score appeared to be a useful outcome parameter in SLE. However, further evaluation in long-term prospective studies is needed.

Concluding remarks

With respect to the questions posed in *Chapter 1* we conclude:

1. Colonization resistance seems decreased in SLE and higher levels of antibodies against autologous bacterial flora are presented in SLE. This is compatible with a role for autologous bacterial flora in the induction and/or activation of anti-dsDNA production in SLE *(Chapter 2 and 3)*.
2. The ^{125}I Farr assay has the highest sensitivity and specificity for detecting antibodies against double-stranded DNA as diagnostic tool for SLE. Serial quantitation of anti-dsDNA levels by this assay can be used. Rises of IgM-class anti-DNA, in contrast to rises in IgG-class anti-dsDNA, are not a sensitive tool for predicting relapses in SLE *(Chapter 4 and 5)*.
3. SLAM and BILAG appeared to be sensitive disease activity measures. The SLICC / ACR damage score is a useful measure of damage *(Chapter 6-8)*.
4. Treatment based on rises in anti-dsDNA prevents relapses in most cases in SLE *(Chapter 7)*.