High Titers of Autoantibodies in Patients with Sickle-Cell Disease

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ABSTRACT. Objective. Frequency and titers of autoantibodies in patients with sickle-cell disease (SCD) have been reported as relatively high. In a prospective study of 88 patients, we examined this “hyper-autoreactivity” and its clinical consequences.

Methods. For 1 year, patients with SCD were screened for the presence in their serum of antinuclear, anti-double-stranded DNA, antiretractile nuclear antigens, anticardiolipin antibodies, and rheumatoid factors. A population of 85 sex-matched individuals of similar ethnic origin served as controls.

Results. Whereas prevalence of autoantibodies did not differ between the 2 groups, the type and rate of antinuclear antibodies were different. Autoantibodies from the SCD patients showed various immunofluorescence patterns, whereas only speckled patterns at low titers were present in controls. No antibody specificity was found in either group. SCD patients and controls displayed similar rates of anticardiolipin antibodies, but the SCD patients tended to be more frequently positive for rheumatoid factors. Six-year followup of the SCD patients did not provide any clinical evidence for onset of an autoimmune disease, except for 1 patient who developed rheumatoid arthritis, with increasing antinuclear antibodies followed by emergence of specific markers 5 years later.

Conclusion. Patients with SCD displayed high titers of autoantibodies. This observation may be due only to immune activation and/or dysfunction in SCD, as neither pathogenic specificity of autoantibodies nor autoimmune clinical signs appeared in the majority of cases in our study. (First Release Dec 1 2010; J Rheumatol 2011;38:302–9; doi:10.3899/jrheum.100667)

Key Indexing Terms:
AUTOANTIBODIES AUTOIMMUNE DISEASE RHEUMATOID ARTHRITIS SICKLE-CELL ANEMIA SYSTEMIC LUPUS ERYTHEMATOSUS INFLAMMATION

Sickle-cell disease (SCD) includes a group of monogenic disorders defined by the production of hemoglobin S. It is characterized by chronic intravascular hemolysis, vasooclusion, and painful episodes that result in damage of multiple organs. Extra-erythrocytic protean manifestations also occur, suggesting the complexity of this molecular disease. Among the extra-erythrocytic manifestations, alterations of the immune system have been highlighted. Immune deficiency and production of autoantibodies have been described in SCD with different frequencies, but they remained clearly higher than in control groups of Africans and Caucasians.

Although rarely reported, autoimmune diseases (AID) occur in adults with SCD, and diagnosis of these can be delayed or missed because of the similarities of their clinical manifestations. This association can severely compromise kidney, heart, bone, lung, or central nervous system functions, as both diseases affect them. To clinically identify the occurrence of an AID in patients with SCD is of critical interest because it requires modification in the therapy. Immunosuppressive treatment has to be undertaken cautiously, as the SCD is likely to be exacerbated in patients with these conditions.

A substantial body of research has shown that populations coming from the same geographical regions as patients with SCD are more likely to develop AID, such as systemic lupus erythematosus, and often with a more severe course, compared to Caucasian people. Recently, the presence of autoantibodies in asymptomatic people have been reported, antedating by many years (3–5 years) the clinical manifestations of AID, and raising the requirement for close clinical and biological followup for early identification of the AID onset.

Based on comparative experiments between patients with SCD and controls of similar ethnic origin, we investigated if...
high prevalence and titers of autoantibodies among SCD patients are common, and if they are an early marker of an ongoing autoimmune process.

MATERIALS AND METHODS

Patients and controls. Eighty-eight patients (54 women, mean age 28.8 years, range 17–50; 34 men, mean age 26.8 years, range 17–55) were recruited into the study over a one-year period (2003). Fifty-five had homozygous sickle-cell disease SS, 25 by compound heterozygote SC, 6 by compound heterozygote SS-thalassemia, one by compound heterozygote S Lepore, and 1 by compound heterozygote SD/Punjab (Table 1). The diagnosis of SCD was made using standard laboratory procedures including a blood count, hemoglobin electrophoresis, and a family study. Sixty-two patients were natives of Africa (West or Central Africa) and 26 came from The West Indies. All patients were living in France but frequently traveled to their country of origin. They were recruited from a cohort of adults with SCD regularly followed at Tenon Hospital, Paris. Samples were obtained during a routine clinical consultation in steady-state, a clinical status characterized by the absence of any infectious process or acute complication (such as vaso-occlusive crisis) in the 3 months preceding the consultation. Patients did not receive transfusions in the preceding 6 months, and had no autoimmunity-inducing therapy. Human immunodeficiency virus, hepatitis C virus, and hepatitis B virus serologies were carried out to exclude patients infected with these autoimmunity-inducing viruses. Pregnant women and patients with known AID were also excluded from the study.

Sex-matched controls consisting of 85 healthy Africans (55 women, mean age 41.5 years, range 24–56; and 30 men, mean age 38.2 years, range 24–56) born and living in Democratic Republic of Congo-Kinshasa (DRC) served as the reference population (Table 1). Their sera samples were previously anonymously collected for a study about prevalence of non-insulin-dependent diabetes (NIDD) in DRC.

The study was approved by the Comité de Protection des Personnes, the ethical research committee of our institution (AP-HP). All patients gave consent for their serum to become part of a sample collection that can be used for further investigations. In return, results were given to the SCD patients and the controls.

After the end of the 1-year inclusion period, all patients were followed clinically for at least 6 years in our hospital. For 7 of them who had high titers of autoantibodies, a biological followup was done periodically.

Methods. Tests for screening of autoantibodies were selected according to the algorithm for AID diagnosis24. At first, antinuclear antibodies (ANA) of IgG isotype were detected on Hep2 cells (Bio-Rad, Hercules, CA, USA) by indirect immunofluorescence (IIF) with a manufacturer’s recommended dilution detection threshold of ≥ 1:50. Significantly high and potentially pathogenic titers are here considered to be ≥ 200 (corresponding to dilution ≥ 1:200). This screening test was followed by detection and/or quantification of specific biologic markers, i.e., anti-double-stranded-DNA (ds-DNA) and antieextractable nuclear antigen antibodies (ENA) SSA, SSB, Sm, RNP, JO1, and Scl70 (Phadia, Immunocap, Uppsala, Sweden). The human and animal rheumatoid factors (RF) were quantified by ELISA (Biomedical Diagnostics, Marne-la-Vallée, France).

The anticardiolipin (aCL) IgG and IgM were screened and quantified by ELISA (Biomedical Diagnostics, Marne-la-Vallée, France) with a positive threshold ≥ 15 U GPL/MPL and a significant rate that had to be taken into account for the antiphospholipid syndrome diagnosis, ≥ 40 U GPL/MPL25.

Positive RF samples with animal and human positivity (thresholds at 30 and 20 IU/ml, respectively) were tested for anticyclic citrullinated peptide antibodies (anti-CCP; Inova, San Diego, CA, USA), a more specific previous marker of rheumatoid arthritis (RA)26.

Subsequently, the ANA-positive samples were tested for antihypertropy oxidative antibodies (Phadia, Uppsala, Sweden) and for anti-smooth muscle, mitochondria 2, liver and kidney microsomes on rat stomach, liver, and kidney sections by IIF to elicit biological markers present in silent autoimmune thyroiditis or hepatic pathology often associated with positive ANA.

Patient and control samples were tested in the same laboratory.

We also investigated the presence of hydroxyurea treatment before the onset of the study, in order to detect any influence of this therapy on the occurrence and/or the rate of autoantibodies.

Statistical analysis. Analysis was performed with chi-square and Fisher’s exact tests using the GraphPad Prism software.

RESULTS

Among the 88 patients with SCD, 43 had detectable ANA (titer ≥ 50) with 17 ≥ 200 compared to 39 and 2, respectively, in the 85 controls. If the frequency of ANA-positive sera did not differ, the SCD patients had a higher rate of autoantibodies than controls (p = 0.0002; Figure 1).

The immunofluorescence aspect showed a wide range of patterns in the SCD patients: speckled, homogeneous, nucleolar, dotted, or others (atypical centromere, etc.; Figure 1), whereas only speckled patterns were found in the control group.

Taking the hemoglobin compound into account, we found no differences among SS, SC, and S80 on the ANA titers and patterns.

There was a higher prevalence for ANA at high titers in the female patients with SCD (p = 0.0003; Figure 2). In contrast to the SCD patients, ANA titers were low, with no obvious female prevalence, in the control group. Indeed, the difference observed in ANA titers between the 2 groups was

Table 1. Characteristics of patients with SCD and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SCD Patients, n = 88</th>
<th>Controls, n = 85</th>
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<tbody>
<tr>
<td>Sex, n</td>
<td>54 women, 34 men</td>
<td>55 women, 30 men</td>
</tr>
<tr>
<td>Age, yrs, mean (range)</td>
<td>Women 28.8 (17–50),</td>
<td>Women 41.5 (24–56),</td>
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<td></td>
<td>men 26.8 (17–55)</td>
<td>men 38.2 (24–56)</td>
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<tr>
<td>Hemoglobin compounds, n</td>
<td>55 SS</td>
<td>85 AA</td>
</tr>
<tr>
<td></td>
<td>25 SC</td>
<td></td>
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<tr>
<td></td>
<td>6 S80</td>
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<td></td>
<td>1 SLepore</td>
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<td></td>
<td>1 SD/Punjab</td>
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</tr>
<tr>
<td>Geographic origin</td>
<td>62 West and Central Africa,</td>
<td>85 Africa, Democratic Republic of Congo-Kinshasa</td>
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<td>26 West Indies</td>
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mainly attributable to women. If we compared the SCD men to the control men, the titers of ANA were not statistically different.

Thirty-six positive aCL IgG with 3 cases ≥ 40 U GPL were found among the 88 patients with SCD, compared to 24 and 1, respectively, among the 85 controls. For aCL IgM, 2 positive with no titer ≥ 40 U GPL were found in the SCD patients versus 4 positive ≤ 40 U GPL in the controls. Statistical analysis revealed no significant difference in the frequency and rates of aCL IgG (Figure 3) and IgM between the SCD patients and controls.

If we look at the influence of the hemoglobin compound on the presence of aCL antibodies, it appears that the SC patients generated aCL antibodies more frequently than the controls (p < 0.05; Figure 3). But differences were not significant between the SS and the SC patients or between the SS patients and the controls.

In contrast to ANA, no difference was seen for aCL positivity and rates among the women.

Five patients displayed positive human and animal RF in the SCD group versus none in the control group. The statistical analysis showed a tendency for the SCD patients to have RF-positive sera more frequently than the controls (p = 0.06; Figure 4).

Despite different panels and titers of autoantibodies between the 2 groups, no specific biomarker of AID (ds-DNA, ENA, CCP) was found in either group. Antithyroperoxodase antibodies were present with positive ANA in a patient with SCD who showed no clinical or biological sign of thyroid dysfunction.

As hydroxyurea is a cytostatic drug that inhibits cell proliferation, we wondered if this treatment, now frequently used in severe cases of SCD, could affect the frequency and rate of autoantibodies. Among the 88 patients, 15 received hydroxyurea before the year of the study. Five developed ANA-positive sera, but all displayed titers ≤ 200 and mainly speckled patterns (Figure 5). When we compared these 15 SCD patients with the rest of the group, a tendency appeared for hydroxyurea-treated patients to be ANA-positive less frequently than nontreated patients (p = 0.053).

We did not find any incidence of the severity of the disease (through the number of hospitalizations of 39 patients...
already followed in Tenon Hospital 1 year before the study) on the occurrence and rate of autoantibodies (data not shown).

The 6-year followup of the cohort did not show any onset of clinical signs of AID in any but one patient with SCD. The titers of autoantibodies remained stable with little or no change in the immunofluorescence pattern. However, for one woman, the ANA titer increased from 100 to 1200 with the occurrence of an anti-RNP specificity, emergence of RF and anti-CCP, and the development of clinical rheumatologic signs that led to a diagnosis of RA.

DISCUSSION

Our study revealed the existence of autoantibodies with

Figure 2. Effects of gender on ANA titers. A. High (≥ 200) and low (< 200) ANA titers in ANA-positive SCD women and in control women. SCD women display higher titers. ***p = 0.0003, chi-square test. B. High (≥ 200) and low (< 200) ANA titers in ANA-positive SCD men and in control men.

Figure 3. Anticardiolipin antibody (ACL) positivity in SCD patients and controls; effect of hemoglobin compound. A. aCL-negative and aCL IgG-positive sera in SCD patients and controls. B. Effect of hemoglobin compound on aCL IgG positivity: frequency of aCL IgG-positive sera is higher in SC patients than in controls. *p = 0.049, Fisher’s exact test.

abnormal rates and patterns (ANA) or frequency (RF) in young adult patients with steady-state SCD in comparison with the control group. It raised the question of their link with either genetic and/or SCD background.

Autoantibodies in young Caucasian people have been reported with low titers, low prevalence (ANA 7%–9%, aCL 4%–6%, and RF 5%–7%), and without higher prevalence in women27,28. In African and Afro-Caribbean subjects, ANA, aCL, and RF have been described with a higher frequency of between 12% and 43% in young healthy people29,30,31. The biological and potential roles of these antibodies are not clearly explained. The difference observed could be linked to genetic factors, but also to environmental exposure. Indeed, as inflammation can trigger the polyclonal activa-
tion of the immune system and autoreactivity, we could link these high rates of autoantibodies in young healthy people living in tropical countries to chronic infections (i.e., malaria, parasitic diseases, tuberculosis, etc.).

To set aside the influence of genetic factors, we chose to compare patients with SCD with sex-matched controls consisting of African subjects coming from the Democratic Republic of Congo-Kinshasa who were healthy or who provided samples for a study about prevalence of NIDD. Unfortunately, we could not undertake age-matching of our 2 populations; we note that the mean age of the control group was older than the SCD cohort. Thus, the differences observed in the titers and frequencies of autoantibodies are likely underestimated. We postulated that the prevalence of AID in NIDD populations is more or less the same as that in non-NIDD populations. The SCD patients included in this study were living in France but were traveling often to their country of origin, where they could be exposed to the same infectious agents as the controls. Even if the environ-

Figure 4. Rheumatoid factor (RF) positivity in SCD patients and controls. SCD patients tend to be more frequently positive for RF than controls. p = 0.06, Fisher’s exact test.

Figure 5. Effect of hydroxyurea treatment on antinuclear antibody (ANA) prevalence, rate, and patterns. A. Number of cases of SCD patients treated or not treated with hydroxyurea that have detectable or undetectable ANA. Detection threshold titer is 50. Hydroxyurea-treated SCD patients tend to be more frequently ANA-positive than untreated patients, p = 0.053, Fisher’s exact test. B. High (≥ 200) and low (< 200) ANA titers in ANA-positive hydroxyurea-treated and untreated SCD patients. C. Distribution of ANA patterns between hydroxyurea-treated and untreated SCD patients.
mental conditions remained relatively different between the 2 groups, epidemiological studies have demonstrated the role of the genetic background as a crucial factor, with a more significant incidence than the environmental exposure, for susceptibility to autoimmune diseases. Indeed, the genetic link between African migrants and Afro-Caribbean people living in the UK remains strong, as they run a similar risk of developing systemic lupus erythematosus (SLE). Finally, while we were aware that our control population did not display exactly similar environmental and genetic backgrounds compared to the SCD patients, it turned out to be the best we could use because of the difficulty of carrying out a case-control study in our situation.

The autoantibodies we observed in the African control group were relatively more frequent (45.8%) compared to those described in young healthy people living in tropical countries. This difference is probably attributable to age. In comparison with the control group, the SCD patients were younger but displayed higher titers of ANA and higher frequency of RF. Altogether, it led us to the question of the direct effects of SCD on the prevalence and rates of autoantibodies.

Autoantibodies were present in a significant number of young patients with steady-state SCD without infections and who had received no transfusions in the preceding 6 months. These patients were asymptomatic, and did not differ from the other SCD patients concerning number of transfusions, the rate and severity of vasoocclusive crisis, and the frequency of documented infections, i.e., occurrence of meningitis, osteomyelitis, pyelonephritis, or septicemia before the time of inclusion to the study (data not shown). Consistent with our results, other studies similarly found no correlations between ANA positivity and disease activity, nor between ANA positivity and number of blood transfusions. Thus, high titers of autoantibodies in SCD patients seem to be correlated to disease status but not severity.

Among the antibodies investigated in SCD, antiphospholipid antibodies (aPL) in particular have been studied because of the phosphatidyl serine exposure, and the shedding of microvesicles induced by the falciformation. Their possible role in thrombotic events has been hypothesized, but discrepancies in methodological assays and fluctuations of these antibodies must be interpreted cautiously. There is often a cross-reaction between antibodies against anionic phospholipids such as phosphatidylserine and cardiolipin. We observed that the SC patients, but not the SS patients, produced more aCL antibodies than the controls. The study included only a limited number of SC patients (n = 25) but we can hypothesize that SC compounds may generate a more significant lipid membrane asymmetry, which could explain the observed differences.

Perhaps RF positivity is only a sign of abnormality in immune complex clearance caused by the spleen dysfunction present in SCD rather than a specific marker of RA. Consistent with this, we found no anti-CCP antibodies in the group of patients with positive RF.

Autoantibodies could be induced by chronic inflammatory conditions as described in cancer. SCD patients displayed chronic innate immunity stimulation by extravascular and intravascular hemolysis. In such a context, with the presence of “danger signals,” autoantibodies against self-components could be produced. Moreover, environmental stimuli, such as recurrent infections in a permanent inflammatory background, are likely to trigger the production of autoantibodies. Indeed, microbial antigens have the potential to initiate autoreactivity through molecular mimicry, polyclonal activation, or release of cryptic antigens.

Another possible explanation for the high production of autoantibodies in SCD patients is the dysfunctional immune status described in the disease, which consists of a functional hyposplenia, a deficit in complement components, an impairment of phagocytosis, a defect in opsonization, and in the clearance of immune complexes. Abnormalities in lymphocyte populations, such as regulatory T cells and natural killer T cells, also could play a role. Some reports suggest that the antibodies against self-antigens found in cancer and during massive tissue damage could have a physiological role, as they could prevent inflammation by facilitating the clearance of oxidized molecules and apoptotic cells. It is possible to extrapolate this other hypothesis to SCD.

Nevertheless, we could not, until now, totally explain the mechanisms by which SCD patients produce such high titers of autoantibodies. Looking at our chronic inflammation hypothesis, the observation that the severity of the disease does not affect the autoantibody rate leaves doubts about its veracity. In contrast, the beneficial effect of hydroxyurea on autoantibody levels is still in favor of an ongoing dysimmune mechanism.

Similarities exist in terms of genetic background, environmental conditions, and immunologic abnormalities in SCD compared to mechanisms of pathogenesis depicted in SLE. In this context, and as other reports have highlighted the presence of autoantibodies antedating onset of AID by many years in asymptomatic populations, we proceeded to a 6-year clinical and biological followup of our SCD patients. It showed no change in their autoimmune profile. Only one of the 43 ANA-positive patients had increasing titers of autoantibodies and emergence of RF, anti-CCP, and anti-RNP specificities that correlated with the appearance of RA clinical symptoms. Thus, whatever the mechanisms of the appearance of autoantibodies in SCD patients, they seem to be quite different from those underlying the development of a true autoimmune process, as both specificity and progression of the autoantibodies were very different.

Nevertheless, even if rarely described in adults with SCD and because of its seriousness, the occurrence of
an AID ought to be taken into account. Physicians have to be alert to unusual complaints and clinical symptoms appearing during the followup of patients with SCD, especially those that can evoke the onset of an AID. A special focus has to be on female patients, and a biological autoimmune examination must be undertaken for early diagnosis of a coexisting AID, in order to institute appropriate therapy and prevent potential complications of this association. In cases when the clinical manifestations are not evocative, immunological investigation does not seem necessary, as ANA positivity with no ENA or ds-DNA specificity or RF presence without CCP positivity is common in SCD.

REFERENCES


