Case Report

De Novo Production of IgG Antinuclear Antibodies in a Neonate

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ABSTRACT. We describe the first case in which *de novo* production of multiple IgG antinuclear antibodies (ANA) occurred in a female neonate of an ANA negative mother. The infant presented at 4 weeks of age with hemorrhagic panencephalitis, diffuse intraparenchymal hemorrhages, and straight sinus thrombosis. She had been vaccinated against hepatitis B at birth. No other cause was found and maternal prenatal care was unremarkable. The infant's screening ANA test by ELISA was positive at 6 weeks with specificity for ssDNA, Sm, and nRNP/Sm. At 8 weeks antibodies to dsDNA and centromere were detected as well. By 8 months, she still had slightly elevated anti-dsDNA, Sm, and nRNP/Sm antibodies. The ANA test by immunofluorescence was abnormal at 8 weeks through 13 weeks with centromere and then homogeneous pattern. Based on similarities with other reported cases, we speculate that hepatitis B vaccination may have been involved in the development of antinuclear antibodies. (J Rheumatol 2001;28:2744–7)

Key Indexing Terms:
ANTINUCLEAR AUTOANTIBODIES INFANCY

Neonates can be primed to produce protective levels of antibodies to recombinant hepatitis B vaccine as early as the first week of life[1]. However, there have been no reported cases of *de novo* autoantibody production in neonates. The earliest detection of autoantibodies in infants was against gliadin and/or endomysium at 7 months of age[2]. All previous reports of autoantibodies against nuclear antigens in neonates have shown that they are passively acquired from the mother and may cause a lupus-like clinical syndrome.

Pediatric systemic lupus erythematosus (SLE) is a rare diagnosis, with an annual incidence of 0.3 to 6 out of every 100,000 children[3,4]; none has been described under the age of 4 years. We describe the first case of a 6-week-old female neonate with *de novo* production of specific IgG antinuclear antibodies (ANA) like those seen in patients with SLE. The clinical presentation was hemorrhagic panencephalitis and straight sinus thrombosis.

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CASE REPORT

Clinical presentation. The patient presented at 4 weeks of life with a 2 day history of fever, poor feeding, stiffness, and eye rolling. The neonate had been vaccinated for hepatitis B at birth and was apparently thriving at her 2 week clinic visit. There was an exposure to a sibling with varicella at 10 days of age. Upon her admission to the pediatric intensive care unit, she was lethargic and toxemic appearing. Her examination was consistent with a catastrophic neurological event. She had no rashes or joint inflammation and urinalysis showed trace proteinuria. Laboratory data from her admission are shown in Table 1. Neuroimaging revealed hemorrhagic panencephalitis with diffuse intraparenchymal hemorrhages and straight sinus thrombosis. Extensive investigations for metabolic or infectious etiologies, including varicella, were unrevealing. In addition, clinical signs of immunodeficiency were absent even after 8 months, when most maternal IgG is decayed. An ANA test was requested at 6 weeks of age and was abnormal (see below). The baby was stabilized and transferred to a subacute care facility at the age of 4 months where she remains.

Serology. ANA were screened by a 2 well ELISA test system, one well containing HEp-2 cell extract and another containing purified antigens, including ssDNA (denatured DNA) (EL-ANAscr, TheraTest Laboratories Inc., Chicago, IL, USA). Positive screens were further tested and quantified by an ELISA specificity panel (TheraTest EL-ANA Profiles, TheraTest Laboratories Inc.). The characteristics of the assays have been described[5]. When first tested at 6 weeks of age, the neonate had abnormal levels of antibodies against ssDNA, Sm, and nRNP/Sm complex. The antibody levels against SSA, SSB, histone, and Scl70 were normal at all times, as were the levels of complement (C3 and C4), antibodies to cardiolipin (EL-ACA, TheraTest Laboratories Inc.), and rheumatoid factor by ELISA (EL-RF/3 IgM, IgG, IgA, TheraTest Laboratories Inc.).

Antibodies to dsDNA became detectable at 9 weeks and were still present at low concentrations when last tested at 8 months of age. In contrast, the mother's serum was negative for ANA (Table 2, Figure 1). The evolution of antibody levels had the character of a "seroconversion," particularly for antibodies against dsDNA and centromere. The anti-dsDNA antibody level started out normal at 6 weeks of age, was abnormal at 9 and 13 weeks, and remained slightly elevated at 8 months of age. It is also worth noting that the increase in anti-ssDNA antibodies preceded that of anti-

Table 1. Analysis of the patient's blood and body fluids at admission (6 weks of age).

Electrolytes in Serum		Complete Blood Count	
Sodium	142 mEq/l	WBC	$17.6 \times 10^3/\mu$l
Potassium	4.6 mEq/l	Neutrophils	24%
Chloride	111 mEq/l	Bands	12%
HCO3	14 mEq/l	Lymphocytes	54%
BUN	13 mEq/l	Hemoglobin	10.4 g/dl
Creatinine	0.5 mEq/l	Hematocrit	30.6%
Glucose	106 mEq/l	MCV	91 fl
		RDW	14
		Platelets	$170 \times 10^3/\mu$l
Urine Analysis		**Cerebrospinal Fluid Analysis**	
Specific gravity	1.018	Appearance	Xanthochromic
pH	5.0	RBC	2266 cells/μl
Protein	100	WBC	200 cells/μl
Ketones	Positive	Neutrophils	43%
Urobilinogen	Negative	Lymphocytes	13%
Blood	Negative	Monocytes	24%
RBC	0	Eosinophils	9%
WBC	0	Glucose	48 mg/dl
Bacteria	None	Protein	852 mg/dl[a]
Culture	No growth	Culture	No growth

BUN: blood urea nitrogen; WBC: leukocytes; MCV: mean corpuscular volume; RBC: erythrocytes; RDW: red cell distribution width. [a] Xanthochromic specimens may falsely elevate results.

dsDNA (a common finding prior to adult SLE flares, unpublished observations). To further determine whether the production of IgG antibodies in the patient followed the natural progression of an active immune response, the IgM autoantibody levels were measured in all of the specimens collected. No abnormal levels of IgM antibodies against any of the nuclear antigens were detected (data not shown).

The evolution of anticentromere antibody levels (recombinant CENP-B antigen; Varelisa, Pharmacia and Upjohn, Kalamazoo, MI, USA) also exhibited a "seroconversion" character. They were normal on week 6 (202 U), became abnormal on weeks 8 (734 U) and 9 (561 U) and returned to normal on week 13 (135 U) and thereafter (Table 2). Concurrent with the appearance of anticentromere, the ANA detected by immunofluorescence (F-ANA™, DiaSorin; Stillwater, MN, USA) became abnormal with a centromere pattern. The antibody titer by F-ANA was normal at week 6 and abnormal at weeks 8, 9 (1:160), and 13 (1:320). The pattern started as centromere at weeks 8 and 9 and became homogeneous at week 13. The F-

Table 2. Autoantibody profiles of the patient and her mother. Normal limits are the upper limits of normal set up by the assay's manufacturers (abnormal results in bold).

Autoantibody Test	Normal Limits (U/ml)	Mother (U/ml)	Autoantibodies (U/ml) in Patient Serum				
			Patient Age at Collection				
			6 wks	8 wks	9 wks	13 wks	8 mo
ANA Screen (well 1/2)[a]	10/10	2/3	13/9	21/11	23/15	13/4	12/5
ssDNA	99	41	177	218	249	237	46
dsDNA	40	21	0	21	120	127	58
Sm	90	11	387	300	424	232	140
Sm/nRNP	83	7	188	132	159	284	123
SSA	91	11	70	37	47	55	68
SSB	73	6	5	0	0	5	34
Histone	96	21	40	51	60	63	34
Scl-70	32	15	0	0	0	9	1
Centromere	300	53	202	714	561	135	89
F-ANA™ [b] titer pattern[d]	≤ 1:80[c]	< 1:80	≤ 1:80	1:160; C	1:160; C	1:320; Hm	< 1:80

[a] ANA screening with 2 wells: one with HEp-2 extract and the other with purified antigens poorly represented in the HEp-2 extract (TheraTest kits).
[b] ANA by immunofluorescence with DiaSorin test kit.
[c] Normal limits have not been established in this age group, but should be ≤ 1:80 (6.2 IU), since the IgG is mainly maternal.
[d] Immunofluorescence patterns: C: centromere; Hm: homogeneous.

ANA antibody titer returned to normal (< 1:80) at 8 months of age. The level of anti-dsDNA detected by *Crithidia luciliae* immunofluorescence (performed by Quest Diagnostics; Wood Dale, IL, USA) was within normal limits in all specimens.

The cognate nature of the interactions between the IgG and the autoantigen on the solid phase was determined by 2 experiments. First, antigen competition for anti-Sm was performed. A solution of bovine Sm antigen (Immunovision, Springdale, AR, USA) in 10 μl of phosphate buffered saline (PBS), or PBS alone as a control, was mixed with 10 μl of patient serum for 30 min at 22°C and diluted in 1 ml of specimen diluent. Controls were similarly prepared using a standard serum containing IgG anti-Sm and a normal serum and were incubated in parallel with the patient sample. Subsequently, the ELISA test was performed according to the manufacturer's instructions (TheraTest). In the presence of the soluble Sm competitor, the antibody activity was reduced by > 90% (data not shown), thereby showing that the reaction was antigen-specific. Second, serum from the patient (8 weeks of age) was tested to identify the presence of anti-dsDNA activity in the $F(ab')_2$ fragments of the IgG, as described[6]. The mother's serum was used as a control. Anti-dsDNA activity was detected in the $F(ab')_2$ fragment of the antibody only in the neonate. This further eliminated the possibility of non-cognate IgG interactions, mainly due to the Fc portion, with selected antigens on the solid phase.

The disease specificity of the tests used was evaluated and the peak F-ANA titer of 1:320 was calculated to be 24.8 IU (normal adult level: 6.2 IU, 95% of adult random blood bank donors). The anti-dsDNA, anti-Sm/RNP, and anticentromere at their peak levels far exceeded the values obtained in 100 random blood bank donors (Figure 1), as well as the manufacturer's established upper limits for the normal adult population (Table 2).

DISCUSSION

We described the first case of *de novo* IgG autoantibody production in a neonate. The antibodies were directed against nuclear antigens, specifically against ssDNA, dsDNA, Sm, and centromere. The period of conversion from normal to abnormal ANA levels was identified between the 6th and 9th weeks of life and displayed the character of a secondary antibody response.

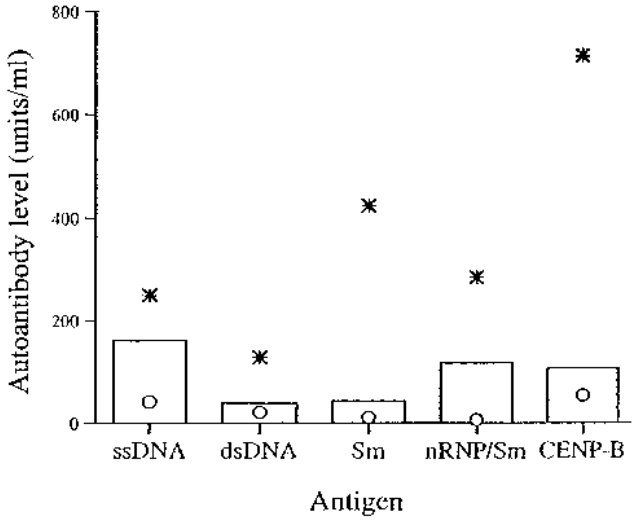

Figure 1. Comparison of the levels of ANA in the patient, her mother, and a random population. Levels of antibodies against the nuclear antigens for which the patient was positive were measured by ELISA; and with the same system, in the patient (highest values obtained with the patient's serum: *), her mother: ○, and in 100 adult blood donors (values for the 99th percentile: □).

Transplacental passage of IgG can be excluded as the source of autoantibody in this neonate, given the normal maternal ANA serology and the pattern of progression of the infant's autoantibody levels. This evolution reflected the characteristics of an active antibody response (seroconversion), most likely a late primary or secondary immune response, since no IgM ANA was detected. The "seroconversion," considered together with the unquestionable maternity, excluded transplacental origin as the source of autoantibodies in this patient.

Other explanations for our findings such as non-cognate binding of the neonate's serum IgG, as that described for the binding of IgG to histone in SLE and rheumatoid arthritis patients[7,8], were very unlikely. First, the binding was directed only against specific antigens (Table 2); second, it was present in the $F(ab')_2$ fragments of the neonate's but not the mother's IgG and most of the neonate's total IgG was of maternal origin; finally, the binding activity was inhibited by free antigen competition.

The neonate had the serologic profile of SLE patients[5]. Moreover, the levels of ANA by F-ANA as well as those for anti-dsDNA, anti-Sm/RNP, and anticentromere antibodies far exceeded those obtained in 100 random blood bank donors (Figure 1). Anticentromere antibodies (Table 2, Figure 1) was detected both by recombinant CENP-B antigen ELISA and by the F-ANA pattern. Although this antibody is mainly a scleroderma marker, it is also found in some patients with SLE[9]. The antibody specificity in this neonate included 2 specific SLE markers, anti-Sm and anti-dsDNA (bacteriophage λ DNA), both detected by ELISA[5]. However, the anti-dsDNA were not detected by *C. luciliae* kinetoplast staining. This is most likely due to the relatively low clinical sensitivity (45%) of the test compared to ELISA (70%) or Farr assay (70%)[5,10]. It is possible that the spectrum of epitopes available for recognition on *C. luciliae* kinetoplast DNA is rather limited compared to the extracted DNA attached to a solid phase (ELISA) or in solution (Farr assay). The recognition of the limited spectrum of epitopes in C. *luciliae* may require further epitope spreading of anti-DNA antibodies during disease progression[11].

The autoantibody levels detected in the neonate's serum were lower than those generally seen in adult SLE patients during flares[5]. This profile, as well as insufficient criteria for SLE diagnosis, suggested that the autoantibodies were more likely to be a consequence of the clinical presentation rather than an association with its etiology. The clinical manifestations met only 3 of 11 versus the necessary 4 American College of Rheumatology criteria, namely, thrombosis, positive ANA, and immunologic abnormality (anti-dsDNA and anti-Sm Ab). In addition, the normal complement levels made SLE an even less likely etiology of the neurological manifestations.

In our patient, the presence of only IgG autoantibodies, without IgM, may have several explanations. First, there

was an early appearance of antibodies, so that only the later part of the primary response, essentially all IgG, was detected by 6 weeks of age. Second, a prior immune response *in utero* may have existed, and the response detected was secondary, and therefore, mainly IgG. Finally, we cannot exclude the possibility of chimerism, with lymphocytes from the mother crossing the placenta and responding to necrotic cells by producing antibodies.

Microchimerism has been described in patients with autoimmune diseases, particularly in those with scleroderma and Sjogren's syndrome[12].

The mechanism that heralds the priming for autoantibody formation as an antigen driven process and its role in SLE is unknown. It has been postulated that tolerance against tissue antigens is maintained when the cells die "naturally" by apoptosis[13]. Alloimmunization and possible autoimmunization may be induced, however, by necrotic cells through the induction of the B7 co-receptor expression by professional antigen presenting cells[13]. Additionally, data obtained from animal experimentation show that tolerance in immunologically privileged sites such as the central nervous system may develop at different windows of time than other sites in the body[14]. This could mean that a breech in the integrity of the blood–brain barrier during the proper time period could also potentially result in the priming of an autoimmune response.

In our patient the underlying cause of autoantibody formation and major neurological disease is a matter of speculation. A possible explanation is that the disease was related to an environmental stimulus, such as hepatitis B vaccination, in a host with a susceptible genetic background. The possibility of an association with hepatitis B vaccination is intriguing. *De novo* onset of SLE and SLE-like syndromes have been reported following hepatitis B vaccination in both adults and children[15-18]. A total of 37 cases have been reported as having a suspected close temporal association with hepatitis B vaccination. The clinical manifestations described are very heterogeneous[18,19] and include practically all of the symptoms seen in SLE[17]; only one patient reported had clinical manifestations similar to our patient's.

In addition, other manifestations have been described, with or without abnormal ANA, and include: erythema nodosum, demyelinization, transverse myelitis, flare of rheumatoid arthritis, central nervous system vasculitis, purpura, retinal vein occlusion[18,19], and even reactive arthritides (Dr. Carol Wallace, Section of Infectious Diseases and Rheumatology, University of Washington, Seattle, WA; personal communication). However, there is insufficient information to determine whether there is a causal relationship. Currently, there is an ongoing multicenter registry across the United States (Dr. Carol Wallace, Section of Infectious Diseases and Rheumatology, University of Washington, Seattle, WA, USA) to document the validity and temporal association of such occurrences.

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