Indirect Immunofluorescence on HEp-2000 Substrate Is a Sensitive Method for Detecting Anti-Ro/SSA Antibodies in Patients with Lupus Erythematosus and/or Photosensitivity

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ABSTRACT. ***Objective.*** To evaluate the sensitivity of indirect immunofluorescence (IIF) utilizing HEp-2000 cells to detect antinuclear and anti-Ro/SSA antibodies.

Methods. We examined 5949 sera samples from 2316 patients with connective tissue diseases. All sera were tested by IIF on HEp-2 cells as substrate and by counterimmunoelectrophoresis (CIE). The positive sera for anti-Ro/SSA antibodies by CIE were retested by IIF using HEp-2000 cells. The sera that were anti-Ro/SSA-positive in CIE but negative on HEp-2000 cells were tested by ELISA for the 60 and 52 subunits of Ro/SSA antigen.

Results. Our results confirm the high sensitivity (81%) of HEp-2000 cells in detecting anti Ro/SSA antibodies.

Conclusion. Using the HEp-2000 substrate is a reliable, simple alternative method for detecting anti-Ro/SSA antibodies in a first-level screening test such as IIF, especially in patients with lupus erythematosus and/or photosensitivity. (First Release June 1 2008; J Rheumatol 2008;35:1320–2)

Key Indexing Terms:
HEp-2000 CELLS
HIGH SENSITIVITY
ANTI-Ro/SSA ANTIBODIES
LUPUS ERYTHEMATOSUS

In dermatology, anti-Ro/SSA antibodies are important because they are the serological marker of subacute cutaneous lupus erythematosus (SCLE), they are present in about 85% of patients with SCLE, and they are often associated with photosensitivity[1,2]. They are usually detected by using second-level methods such as counterimmunoelectrophoresis (CIE), enzyme linked immunosorbent assay (ELISA), or immunoblotting assay. The detection of Ro/SSA antibodies by indirect immunofluorescence (IIF) on conventional HEp-2 cells used as a substrate to detect antinuclear antibodies (ANA) lacks sensitivity.

As the possibility to simultaneously detect ANA and anti-Ro/SSA antibodies by IIF is appealing, an alternative substrate has been proposed[3]. We show here that anti-Ro/SSA antibodies are easily identifiable using HEp-2000 cells, a commercially prepared substrate in which about 10%–25% of cells are transfected with multiple copies of the cDNA that expresses the 60 kDa subunit of the Ro/SSA antigen[4]. Anti-Ro/SSA are characterized by a distinct bright speckled pattern with prominent staining of 10%–25% of the interphase nucleoli (Figure 1).

MATERIALS AND METHODS

We examined 5949 consecutive sera samples collected over 5 years (2000–2005) from 2316 patients with connective tissue diseases, in particular lupus erythematosus (LE) with prevalent cutaneous lesions such as localized and disseminated discoid lupus and SCLE. Photosensitivity was reported by all patients with SCLE and by about half of the other patients with LE. For each patient, more than 1 serum was tested (average 3 samples for each patient). All the examinations were requested routinely by clinicians.

All sera were tested by IIF by using HEp-2 cells as substrate (ImmunoConcepts, Sacramento, CA, USA, distributed by Technogenetics Bouty Group, Milan, Italy) at the dilution of 1/40. The dilution of 1/40 is low and often it can give a high percentage of false-positive ANA; however, we usually use this dilution to be sure to detect any antibodies present. The sera were also tested by CIE using standard protocol with calf spleen and rabbit thymus acetone powder extracts as sources of Ro/SSA-Sm and La/SSB ribonucleoprotein (RNP) autoantigens[5]. All the sera that proved to be positive for anti-Ro/SSA antibodies by CIE were retested by IIF using Ro/SSA transfected HEp 2 (HEp-2000) cells (ImmunoConcepts; imported by Technogenetics Bouty Group). The assay was performed according to the manufacturer's instructions.

In addition, the sera that were anti-Ro/SSA-positive by CIE but negative on HEp-2000 cells were tested by ELISA using 2 commercial kits: anti-SSA 60 (Orgentec Diagnostika GmbH, Mainz, Germany) and anti-SSA 52 (Orgentec; imported by Technogenetics Bouty Group). These 2 ELISA are used for the quantitative measurement of IgG class autoantibodies to the Ro/SSA 60 and 52 subunits of Ro/SSA antigen in human sera.

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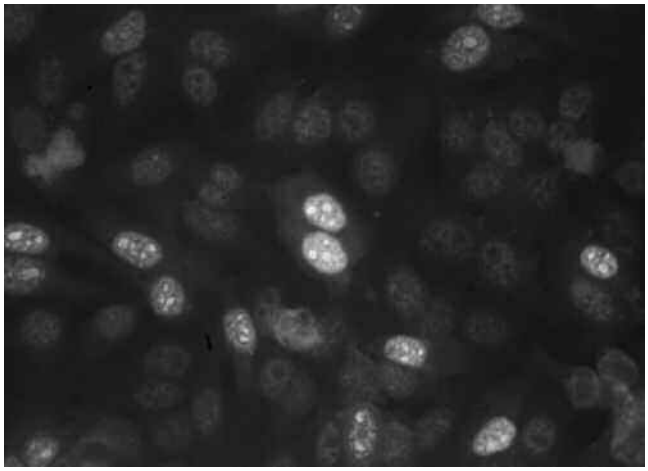

Figure 1. Distinct bright speckled pattern with prominent staining of the nucleoli in 10%–20% of the interphase nuclei, while the chromosome region of metaphase mitotic cells is negative.

RESULTS

Of the 5949 sera, 434 (7%, belonging to 118 patients) proved to be positive for anti-Ro/SSA antibodies by CIE. Three hundred twenty-three were from patients with LE, 6 from patients with Sjögren's syndrome (SS), and 107 from patients with other connective tissue diseases. In addition, in 92 sera from patients with LE, 4 from SS, and 23 from other connective tissue diseases, CIE detected anti-La/SSB antibodies as well.

When tested on HEp-2000 cells, 90 of the 118 patients (76%) had the typical anti-Ro/SSA IIF staining pattern, but 25 exhibited other IIF staining patterns and 3 were negative. In 2 of the 25 the antibody titers were low, and in 23 they were ≥ 1/160. Three exhibited a nucleolar pattern, 4 a homogeneous pattern, and 18 a speckled one. Using HEp-2 nontransfected cells as substrate, 8 patients showed negative results and 17 positive speckled ANA (final titer 1/40). Among the 28 sera that were positive by CIE and negative on HEp-2000 cells, 26 were positive by ELISA, 17 to the 60 kDa protein, 1 to the 52 kDa protein, and 8 to both (Table 1). Twenty-seven of these patients had LE and 1 systemic sclerosis.

DISCUSSION

HEp-2000 cells are an advanced IIF substrate for detection of ANA, with a high specificity and a great sensitivity for anti-Ro/SSA antibodies. In this substrate, about 10%–25% of the cells are transfected with multiple copies of the spe-

Table 1. Patients with positive CIE for anti Ro/SSA antibodies and negative IIF on HEp-2000 for anti Ro/SSA specificity.

Patient	CIE Ro/SSA + Other Antibodies	IIF on HEp-2000	ELISA 52-60 Ro/SSA 52 kDa Subunit, Cutoff 1.1	ELISA 52-60 Ro/SSA 60 kDa Subunit, Cutoff 0.44
1	Pos	Neg	Neg	Pos (1.65)
2	Pos	1/320 homogeneous	Pos (2.92)	Pos (3.31)
3	Pos	1/1280 nucleolar	Pos (2.54)	Pos (2.91)
4	Pos	1/640 speckled	Pos (2.03)	Pos (1.03)
5	Pos	1/1280 homogeneous	Neg	Pos (2.38)
6	Pos	1/640 speckled	Pos (1.35)	Pos (3.06)
7	Pos	1/1280 speckled	Pos (2.72)	Pos (3.19)
8	Pos + La/SSB	1/320 speckled	Neg	Pos (2.25)
9	Pos + La/SSB	1/1280 nucleolar	Pos (2.94)	Pos (3.18)
10	Pos	Neg	Neg	Pos (1.67)
11	Pos	1/640 speckled	Neg	Pos (2.76)
12	Pos + La/SSB	1/320 speckled	Pos (2.00)	Neg
13	Pos	1/40 speckled	Neg	Pos (1.22)
14	Pos	1/2560 homogeneous	Neg	Pos (3.21)
15	Pos	1/320 speckled	Neg	Pos (1.34)
16	Pos	Neg	Neg	Pos (1.29)
17	Pos + La/SSB	1/40 speckled	Neg	Neg
18	Pos	1/640 speckled	Pos (2.03)	Pos (2.72)
19	Pos	1/160 speckled	Neg	Pos (2.49)
20	Pos + La/SSB	1/320 speckled	Neg	Pos (1.86)
21	Pos	1/640 nucleolar	Neg	Neg
22	Pos + La/SSB	1/1280 speckled	Pos (2.87)	Pos (3.08)
23	Pos	1/160 speckled	Neg	Pos (2.06)
24	Pos	1/160 speckled	Neg	Pos (2.56)
25	Pos	1/160 speckled	Neg	Pos (1.40)
26	Pos	1/320 homogeneous	Neg	Pos (0.87)
27	Pos	1/640 speckled	Neg	Pos (2.22)
28	Pos	1/160 speckled	Neg	Pos (0.48)

cific DNA sequence that carries the information for the 60 kDa Ro/SSA autoantigen[6]. As sera from patients with LE usually recognize the 60 kDa subunit[6], the importance of such substrate for dermatologists is evident, because in patients with LE and cutaneous lesions anti-Ro/SSa antibodies are quite frequent[1]. Using this substrate in IIF we cannot detect antibodies directed to the 52 kDa subunit of Ro/SSA antigen; however, anti-52 kDa Ro/SSA antibodies as the only antibody are relatively uncommon in SLE, SCLE, and SS[7,8]. For its detection a second-level test is needed.

Our results confirm the high sensitivity (81%) of HEp-2000 cells, according to the Altman test[9], in detecting anti-Ro/SSA antibodies. This sensitivity is as high as that described in the literature[4,6,10-13]. Only 28 patients positive by CIE did not show the typical pattern of anti Ro/SSA antibodies, possibly because of the high titers of other autoantibodies that masked the speckled/nucleolar pattern typical of anti-Ro/SSA antibodies.

The great majority (95%) of our patients had LE with prevalent cutaneous lesions, most of them reporting photosensitivity, or SCLE and they are anti-Ro/SSA-positive very often, as much as 80%–90% in SCLE.

Our results confirm that using HEp-2000 substrate is a reliable and simple alternative method for detecting anti-Ro/SSA antibodies in a first-level screening test such as IIF, especially in patients with LE and/or photosensitivity[2,14,15].

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