Evaluation of Cellular Substrates for Antinuclear Antibody Determinations

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The immunofluorescent technique was employed to evaluate the sensitivity of 10 human and animal cell monolayers and tissue sections as substrates for titrating the antinuclear antibody content of serum samples. The highest mean ranks of sensitivity, the relative ability of each substrate to maintain its sensitivity rank when 21 selected positive sera were tested, were achieved by two fibroblast cell lines, baby hamster kidney (BHK 21/C13) and human lung (WI-38), respectively. The least sensitive substrates were commercial rat kidney and liver tissue sections.

Although antinuclear antibodies (ANA) may be demonstrated by many different methods (3), the immunofluorescent procedure has been used most widely. That a variety of nuclear substrates are employed in this procedure (1) may account, in part, for the differences experienced in detecting ANA, reproducing serum titration end points, and discerning immunofluorescent nuclear patterns. Because the amount of deoxyribonucleic acid per cell nucleus varies in different tissues as well as species (8), these discrepancies in ANA testing may be related to antigen-antibody ratios which may be less than optimal when cells of diverse tissues are used.

Cell monolayers have been employed successfully as substrates in ANA determinations (4, 5). For discerning immunofluorescent patterns they have been used to locate precisely the reactions between ANA and nuclear components (6). Cell monolayers, particularly of an established cell line, may provide a sensitive and reproducible substrate acceptable as a standard in ANA tests. In this present study, an attempt was made to evaluate the sensitivity of a variety of human and animal cell monolayers and tissue sections by using these substrates to titrate selected serum samples for ANA content.

MATERIALS AND METHODS

Cell substrates. Cell monolayers of test substrates (Table 1), with the exception of those obtained from commercial sources, were propagated in eight-chamber unit slides (Lab-Tek Products, Westmont, Ill.) with Eagle minimal essential medium containing 10% fetal calf serum. Cell suspensions containing approximately 10^6 cells per 0.4 ml were added to slide chambers which were then incubated

at 35 C for 24 h. Cell monolayers were rinsed once with 0.01 M phosphate-buffered saline, pH 7.2, fixed with 95% ethyl alcohol, and stored at -70 C for as long as 4 weeks. Before use in ANA tests, cell monolayers were rinsed with phosphate-buffered saline.

Sera. Twenty-one human serum specimens, positive for ANA, were obtained through the courtesies of F. P. Quismorio, Los Angeles County/University of Southern California Medical Center, Los Angeles, Calif.; C. E. Buckley, Duke University Medical Center, Durham, N. C.; Robert F. Ritchie, Maine Medical Center, Portland, Me.; and the West Virginia University Medical Center, Morgantown, W. Va.

Conjugate. Commercial fluorescein-conjugated goat antiserum to human immunoglobulin G (Meloy Laboratories, Springfield, Va.) was used in all tests at a dilution of 1:10.

ANA determinations. For evaluation of substrate sensitivity, each of 21 ANA-positive serum specimens as well as five normal sera were tested for ANA content on 10 different cell substrates, using the indirect immunofluorescent procedure. From an initial 1:10 dilution, twofold dilutions of serum specimens were prepared in phosphate-buffered saline and added in 0.5-ml volumes onto cell monolayers or tissue sections. For each serum sample, one set of dilutions was prepared and tested on the same day on all 10 substrates. After incubation at room temperature for 30 min, cell substrates were rinsed three times with phosphate-buffered saline. Diluted conjugate in a 0.4-ml volume was applied to each cell substrate and the incubation and rinsing procedures were repeated. Cell substrates were then mounted under cover glass slips with a semipermanent medium (7). Appropriate controls consisting of known positive and negative serum specimens were included in each test with all substrates.

Cell substrates were examined with a Zeiss fluorescence microscope, equipped with a FITC exciter and no. 50 and 65 barrier filters, at a magnification of $\times 125$ or $\times 200$. The ANA titer of test serum was

TABLE 1. *Description of cell substrates*

Cell line	Origin
WI-38	Normal human embryonic lung, diploid
HeLa	Human epitheloid carcinoma of cervix, heteroploid
HEp-2	Human epidermoid carcinoma of larynx, heteroploid
1-5c-4	Wong-Kilbourne derivative (D) of Chang human conjunctiva, heteroploid
E-N ^a	Human epithelial cells, origin unknown
BHK 21/C13	Syrian baby hamster kidney, heteroploid
LLC-MK ₂	Rhesus monkey kidney, heteroploid
L-929	Clone of strain L, mouse connective tissue, heteroploid
RK ^c	Rat kidney sections
RL ^c	Rat liver sections

^a Commercial substrates. Remaining cell lines were obtained from American Type Culture Collection, Rockville, Md.

the reciprocal of the highest dilution that produced fluorescent staining of nuclei.

Statistical evaluation. The Friedman test (2) with $\alpha = 0.05$ was used to validate the sensitivity of cell substrates for assessing the ANA content of serum samples. The 10 substrates were ranked from 1 (low) to 10 in accord with ANA titers obtained for each serum. Equal titers were each given a value calculated from the mean of the corresponding range of ranks. Substrate sensitivity was expressed as the arithmetic mean of the rank values, i.e., the relative ability of each substrate to maintain its rank in sensitivity when 21 different sera were tested. Substrates were placed in sensitivity groups and the probability that the substrate of a group is significantly ranked in that group was determined.

RESULTS

The 10 cell substrates tested in ANA titrations of serum samples are listed in order of their decreasing sensitivity (Table 2). The baby hamster kidney (BHK 21/C13) cell line achieved the highest mean rank and was the most sensitive of the tested substrates. Next in sensitivity was the cell line WI-38. Both were fibroblast cell lines, and each of them comprised significantly discrete sensitivity groups. The remainder of the human cell substrates formed the third group. The animal cell lines L-929 and LLC-MK₂ comprised the fourth group. The least sensitive of the tested substrates, achieving the lowest rank, were commercial rat kidney and rat liver tissue sections. The ANA titers of the 21 positive test sera ranged from 1:40 to 1:51,200.

Representative examples of fluorescent nuclei in different cell substrates are shown in Fig. 1. In all cell monolayers, each serum sample induced a consistent pattern of nuclear fluorescence, i.e., speckled, peripheral, or homogeneous, which did not change with serum dilutions. Although these patterns were easily discernible in cell monolayers, they were difficult to visualize in rat kidney and rat liver tissue sections. With all substrates, no sign of nuclear fluorescence was noted when normal serum samples were tested.

DISCUSSION

Although the scope of this study was limited to an evaluation of 10 cell substrates, the findings indicate that different cell monolayers vary in their sensitivity for detecting ANA in serum samples. The highest mean ranks, indicative of greater sensitivity, were achieved by BHK 21/C13 and WI-38 cell monolayers, respectively. Most of the human cell monolayers comprised a third distinct sensitivity group; cell monolayers or animal origin formed the fourth group.

Although BHK 21/C13 and WI-38 cells differ in their origin and karyotype, fibroblast morphology is a feature common to both. The significance of this factor in relation to the greater

TABLE 2. *Mean ranks of substrates in their sensitivity for ANA determinates of 21 serum samples^a*

Cell substrate ^a	Mean rank ^b	Sensitivity group (probability) ^c
BHK 21/C13	9.31	I (<0.01)
WI-38	7.48	II (<0.05)
HEp-2	6.52	III (<0.05)
HeLa	6.50	III (<0.05)
E-N	6.48	III (>0.05) ^d
1-5c-4	5.21	IV (>0.05) ^e
L-929	5.12	IV (<0.01)
LLC-MK ₂	4.00	IV (<0.01)
RK ^f	2.31	V
RL ^f	2.07	V

^a ANA titer of each serum sample was determined on 10 test substrates.

^b Listed in order of decreasing sensitivity.

^c Ranking scale 1 to 10: 1, lowest titer; 10, highest titer. Equal titers given an average rank.

^d Probability that the substrate of a group is significantly ranked in that group.

^e Cell substrate is not significantly different from L-929 nor from HeLa.

^f RK, Rat kidney; RL, rat liver.

FIG. 1. Nuclear fluorescence in different cell substrates using an identical dilution of one ANA-positive serum sample. A, WI-38; B, 1-5c-4; C, LLC-MK₂; D, HEp-2; E, E-N; F, BHK 21/C13; G, HeLa; H, L-929; I, RK. $\times 125$.

sensitivity of these cell lines for ANA determinations when compared to other cell substrates of diverse origin, karyotype, and morphology is uncertain. Varied nuclear protein concentrations of different composition may be found in diverse cell substrates (8) which could account for the range of cell sensitivities observed.

The least sensitive of the tested substrates, achieving the lowest rank, were commercial rat liver and rat kidney tissue sections. Nuclear patterns of fluorescence in these substrates were also difficult to discern. The poor results obtained with these commercial tissue sections does not imply that all tissue sections are inferior to cell monolayers for ANA determinations. The use of tissue sections, freshly and properly processed from suitable hosts, in all probability, may be comparable to cell cultures in many respects, i.e., sensitivity and recognition of nuclear patterns.

As a standard substrate for detection and titration of ANA, established cell lines offer the advantages of universal availability from cell repositories, homogeneity of cell population, ease of discerning nuclear fluorescence pat-

terns, sensitivity, and long-term storage potential of unstained cells. Further evaluation of established cell lines for ANA detection may reveal others, comparable to or more sensitive than those cell lines presently described.

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