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## The Pseudo-Leukocyte-Specific/Nuclear Membrane Antinuclear Antibody Pattern: A Puzzle

Thomas K. Burnham, MB, BS\* (London)

*We report an antinuclear antibody (ANA) pattern—the pseudo-leukocyte-specific/nuclear membrane (PLS/NM) ANA pattern—that is puzzling because it usually reacts with only a few cells on human spleen imprints but with most epidermal and numerous dermal nuclei of normal human skin sections. The designation “pseudo-leukocyte-specific” was chosen because it is generally seen in only a few nuclei on human spleen imprints, thereby resembling the true leukocyte-specific ANA pattern. A few small round nuclei*

*fluoresce homogeneously, surrounded by a bright wall, suggesting nuclear membrane (NM) fluorescence. The term “nuclear membrane” was added to the name because the NM appearance is even more prominent on skin sections where the pattern can be mistaken for the peripheral ANA pattern of systemic lupus erythematosus (SLE). However, the PLS/NM pattern has no clinical specificity, which makes it essential to recognize it as separate from the pattern of peripheral fluorescence.*

For over 15 years, during which time we tested the sera of approximately 38,000 patients (Table I), we have seen a pattern of nuclear immunofluorescence that is puzzling because it usually reacts with only a few cells on human spleen imprints but with most epidermal and numerous dermal nuclei of normal human skin sections. Because of our uncertainty regarding the mechanism of this type of nuclear immunofluorescence, we did not include it in our previous descriptions and clinical correlations of the antinuclear antibody (ANA) immunofluorescent patterns (1-10). However, since we see this pattern fairly frequently, we wish to report it for the sake of completeness and in the hope that this report might stimulate further research into the puzzle.

The designation “pseudo-leukocyte-specific” (PLS) was chosen because on human spleen imprints generally only a few nuclei demonstrate this pattern, thereby resembling the true leukocyte-specific antinuclear antibody pattern (LSANA) (11,12). It is a nonparticulate pattern that, unlike the particulate patterns (7), is not composed predominantly of stained particles.

The morphology of this pattern is homogeneous, surrounded by a bright wall, suggesting nuclear membrane involvement. Because the nuclear membrane appearance is even more prominent on skin sections, we added the term “nuclear membrane” (NM) to the name. We report the morphology, nuclear reactivity, and lack of clinical specificity of the PLS/NM ANA pattern.

### Materials and Methods

#### Clinical material

Sera of patients who had connective tissue diseases, miscellaneous medical diseases, malignancies, and miscellaneous dermatoses were studied (Table I). Diagnoses were based on characteristic clinical features and confirmatory laboratory results.

#### Antinuclear antibody testing

The immunofluorescent spleen imprint technique was employed (1,2). This technique uses touch imprints of surgically obtained human spleens as nuclear substrate for the indirect fluorescent antibody technique. The spleens were initially frozen in liquid nitrogen and stored at  $-76^{\circ}\text{C}$ . Commercially available fluorescein-conjugated goat antihuman gamma globulin (Baltimore Biological Laboratories) was used (1).

Sera were titrated undiluted 1:2, 1:5 and then diluted at twofold dilutions in veronal buffered saline (pH 7.2 to 7.3). The highest dilution producing an unequivocally positive result followed by two consecutive negative dilutions was considered to be the titration end point (1).

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## Pseudo-Leukocyte-Specific ANA

**Table I**  
**Incidence and Titers of the PLS/NM ANA Pattern Since First Recognized 11/1/69 Through 3/31/85**

	Total Patients Tested for ANA	Blood Donors	RA	SLE	DLE	Scleroderma
Number of patients tested	38,000	100	1,544	543	373	383
Number and percentage ( ) positive for PLS/NM ANA without associated other ANA pattern	236 (0.62%)	0	37 (2.4%)	0	4 (1.07%)	3 (0.78%)
Number and percentage ( ) positive for PLS/NM ANA with and without associated other ANA pattern	273 (0.72%)	0	47 (3.0%)	3* (0.55%)	4 (1.07%)	3 (0.78%)
Titer range and median titer values ( ) of patients positive for PLS/NM ANA without associated other ANA pattern	U <sup>†</sup> -320	-	U-320 (20)	-	2-20 (5)	2-5 (5)

\*1 definite SLE, 2 probable SLE

<sup>†</sup>U = positive undiluted only

The slides were examined without cover slips with an Ortholux II fluorescence microscope with a 95 X fluorite oil-immersion objective with transmitted and incident light.

### Other nuclear substrates

Selected sera demonstrating the PLS/NM ANA pattern were tested on human spleen sections, human normal skin sections, and human tonsil imprints, which were initially frozen in liquid nitrogen. They were also tested on human buffy coat peripheral blood smears (initially frozen and fresh) and human Hep-2 cells (not frozen) (Table II).

All these substrates were tested with the antigamma globulin conjugate except for the skin sections. The skin sections were tested with fluorescein-labeled goat antihuman IgG and anti-C<sub>3</sub> with the conjugate characteristics and procedure as previously described (13-17). Sera were titrated on the skin sections with the anti-IgG conjugate. The sera were tested undiluted, diluted 1:10, and then (sometimes) at twofold but more usually at fourfold dilutions. Sera from 51 patients were titrated both on human spleen imprints with the antigamma globulin conjugate and on the human skin sections with the anti-IgG conjugate.

### Evaluation of different conjugates regarding comparative morphology and titers of the PLS/NM ANA pattern

Sera producing this pattern were tested on the human spleen imprints with the anti-IgG and anti-C<sub>3</sub> conjugates that were used on the skin sections as well as with the antigamma globulin conjugate normally used on the spleen imprints for comparative morphology and evidence of C<sub>3</sub> binding.

Simultaneous titrations of sera from ten patients in whom this pattern was seen were performed on the spleen imprints with the anti-IgG and antigamma globulin conjugates. The same sera were titrated with the anti-IgG conjugate on the skin sections within one to 16 days of the spleen titrations. The comparative titrations were performed to ensure that any differences or similarities in titers that might be found on the skin sections vs the spleen imprints were not due to the usual employment of the anti-IgG conjugate on the skins and the antigamma globulin conjugate on the spleen imprints.

### Blocking procedures

Blocking procedures using sera demonstrating this pattern were performed on human spleen imprints and normal

**Table II**  
**Other Human Nuclear Substrates Tested with Sera of Patients with the PLS/NM ANA Pattern on Human Spleen Imprints**

	Skin	Blood Smear	Tonsil	Hep-2 Cells	Spleen Section
Number of patients tested and positive ( ) for PLS/NM ANA	80 (80)	14 (0)	4 (4)	4 (0)	2 (2)

human skin sections. Sera were diluted up to twofold dilutions below their original titer and were incubated with these two substrates followed by unlabeled antihuman IgG and then the conjugated anti-IgG. Control slides were incubated with bovine albumin after the serum incubation instead of with the unlabeled antihuman IgG followed by the conjugated anti-IgG. This represents a control incubation with another protein solution instead of the unlabeled antihuman IgG. Blocking could only be confirmed if the slides incubated with the bovine albumin were positive while those incubated with the unlabeled antihuman IgG were negative.

#### Antideoxyribonucleic acid tests

Tests for IgG antibodies to single-stranded deoxyribonucleic acid (DNA) were performed on sera from 40 patients with this pattern. A solid-phase radioimmunoassay (RIA) technique (18) was used.

#### Antiextractable nuclear antigen tests

Tests for antiextractable nuclear antigen (ENA) antibodies

were performed on sera from the same 40 patients tested for single-stranded anti-DNA antibodies. A modification of the technique of Nakamura et al (19) was employed.

## Results

#### Clinical correlations

There have been no specific diagnostic correlations of this pattern. The pattern may be seen in any of the connective tissue diseases, with the highest incidence found associated with rheumatoid arthritis. Conversely, no patients in whom a diagnosis of systemic lupus erythematosus (SLE) was confirmed demonstrated this nonparticulate pattern (7) without an associated other ANA pattern (Table I). This is consistent with our previous findings (20) that indicated that patients who have SLE and other nonparticulate patterns (7), such as the peripheral and homogeneous patterns, usually have concomitant particulate patterns (showing stained particles) (7,20). The only three patients with SLE who had this pattern (Table I) had associated particulate patterns. One of the two patients with probable SLE had only a questionable concomitant particulate pattern.

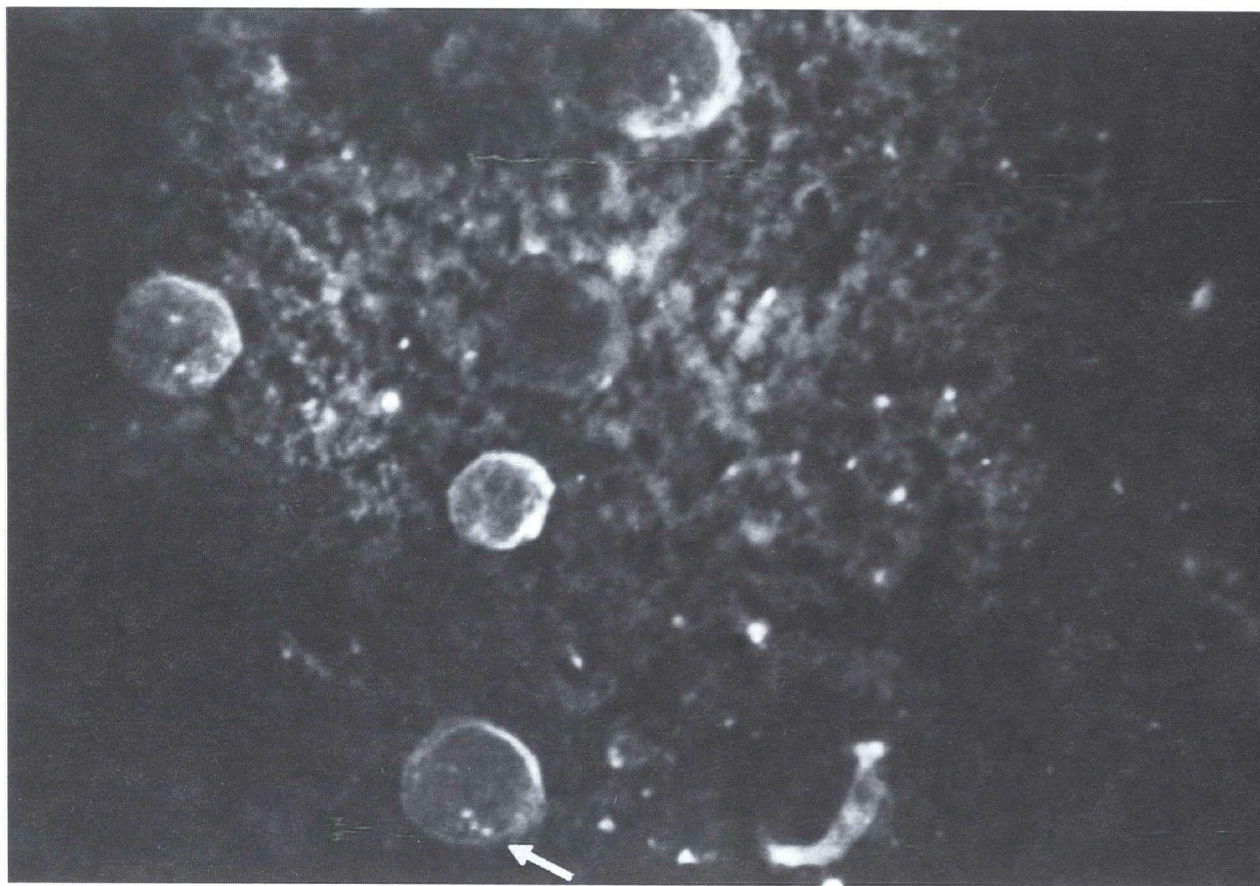


Fig 1

Pseudo-leukocyte-specific/nuclear membrane (PLS/NM) antinuclear antibody (ANA) pattern on human spleen imprint. Scattered small round nuclei are partially (arrow) and completely surrounded by a brightly fluorescent nuclear membrane-like wall. Scattered brightly fluorescent, stippled components overlie the bodies (arrow) and walls (upper left nucleus) of these nuclei. Original magnification x 320.

## Pseudo-Leukocyte-Specific ANA

The PLS/NM ANA pattern was also seen in patients who had malignancies and miscellaneous medical diseases including non-SLE renal diseases. It was found in three patients who had poststreptococcal glomerulonephritis. However, none of 100 blood donor control subjects showed this ANA pattern. Positive results with the anti-C<sub>3</sub> conjugate were also sometimes seen, but again there was no association with any specific disease.

No distinctive clinical characteristics were noted in the four patients who had discoid lupus erythematosus (DLE) and the three patients who had scleroderma in whom this ANA pattern was the only pattern.

Titers (Table I) were relatively low (range: positive undiluted only to 320); the majority of patients had titers of 40 or lower. There was also no significant correlation between titers and specific diagnoses, although the median titer was 20 in cases of rheumatoid arthritis, which was slightly higher than in cases of DLE and scleroderma (where it was only five). Titers of 40 were seen in patients who had ulcerative colitis and poststreptococcal glomerulonephritis. One

patient who had mild alcoholic cirrhosis and degenerative joint disease had a titer of 80.

### PLS/NM ANA pattern on human spleen imprints and spleen sections

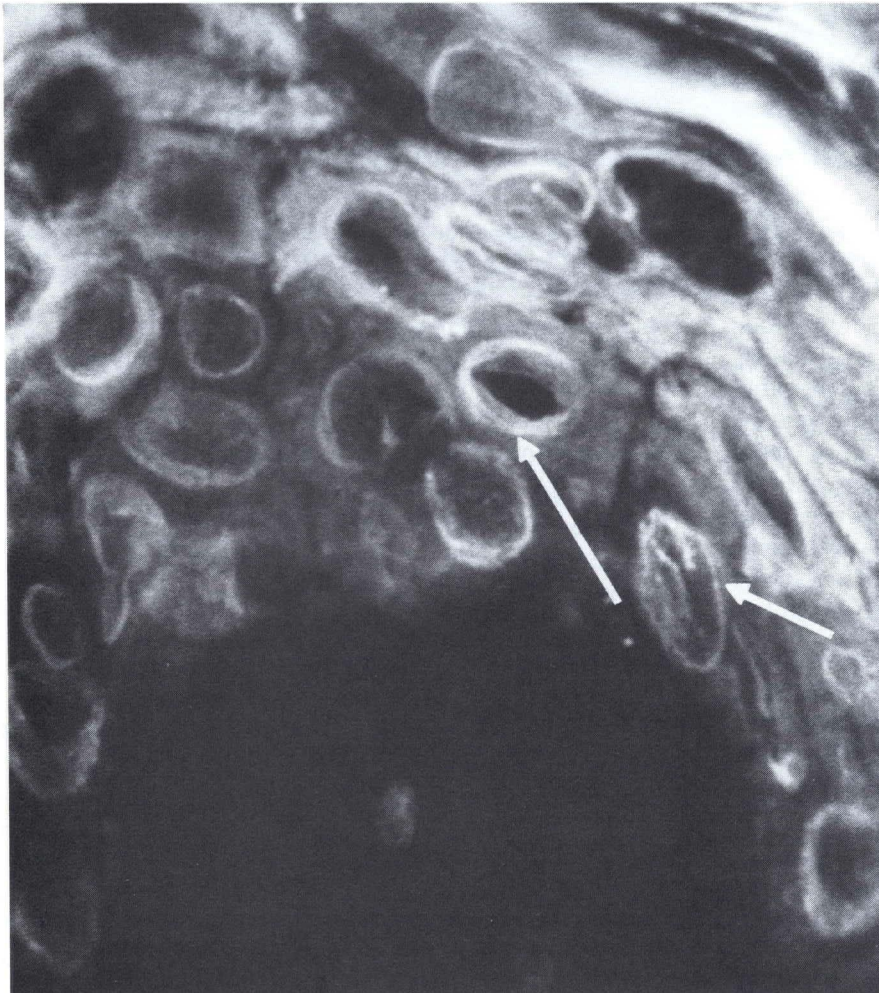
This pattern was characterized on imprints by generally involving only a few randomly distributed nuclei. The cells appeared small and round, and the center of the nucleus showed homogeneous fluorescence of various intensities surrounded by a bright wall that sometimes showed a dark center within it. Sometimes a few stippled structures were seen overlying the walls and centers of the nuclei (Fig 1).

The appearance on human spleen sections was similar to that on imprints in that again only randomly scattered positive nuclei were seen. There was no tendency for positive nuclei to be localized to any specific anatomic sites.

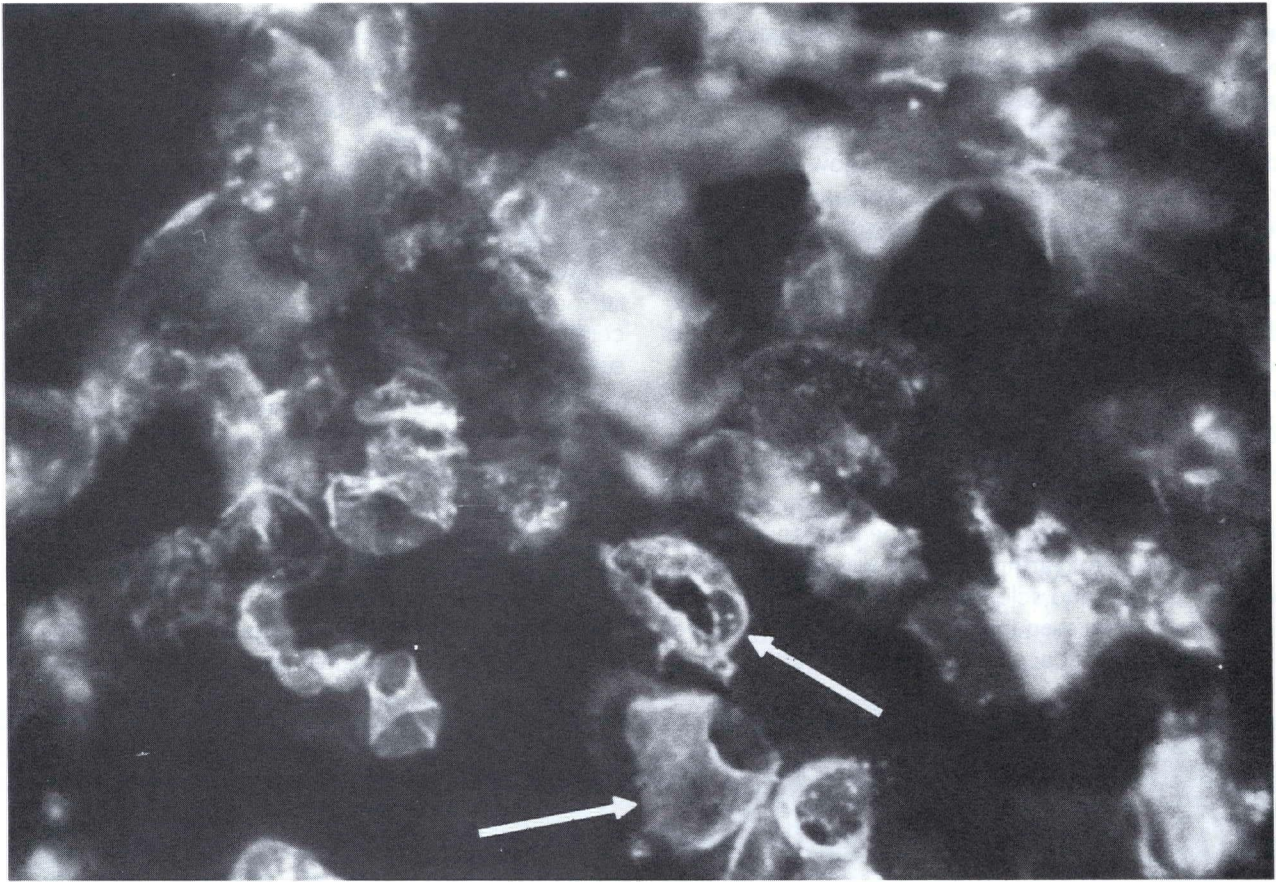
### PLS/NM ANA pattern on other nuclear substrates

#### *Human normal skin sections*

The morphology differed slightly on the skin sections (Figs 2 and 3) (Table II) from that on the spleen imprints in that



**Fig 2**  
PLS/NM ANA pattern on human normal skin section. Most epidermal nuclei including those of basal cell layer (right arrow) and few in dermis (lower part of figure) show this pattern. Note NM-like, dark-centered walls that are separated by wide gaps consisting of cytoplasm and intercellular spaces. Stippled components are seen overlying some of these walls (arrows) including one basal cell nucleus (right arrow). Original magnification x 320.



**Fig 3**  
**PLS/NM ANA pattern on human normal skin section. Several dermal nuclei show brightly fluorescent, dark-centered walls. Bright stippled components overlie the walls (upper arrow) and centers of some nuclei. Note that some walls can be very broad (lower arrow). Original magnification x 320.**

the NM-like appearance was more prominent. There tended to be less central homogeneous fluorescence possibly because these were sections rather than imprints. The other minor difference was that the stippled components overlying both the nuclear walls and centers were seen more frequently on the skin sections than on the spleen imprints. The striking difference was one of numbers of positive nuclei rather than morphology. The majority of epidermal nuclei showed this pattern in all parts of the epidermis from the basal cell layer to the granular layer. Numerous positive nuclei were also seen in the dermis (Fig 3).

Despite the generally greater number of positive nuclei in skin sections compared with the number in spleen imprints, no significant differences were found between titers on the skin sections and the spleen imprints of the 51 patients.

The PLS/NM ANA pattern on skin had to be differentiated both from peripheral ANA (Fig 4) and the general epidermal cytoplasmic (GEC) patterns (15-17). It differed from the peripheral ANA pattern in that the fluorescent nuclear walls tended to be broader than the usually thinner appearing walls of the peripheral pattern. The PLS/NM ANA pattern

could be further differentiated both from peripheral ANA and GEC by the presence of wider gaps between the fluorescent walls. The gaps were composed of cytoplasm and intercellular spaces (Fig 2). Also more fluorescent nuclei were found in the dermis than are found with GEC.

#### *Human tonsil imprints*

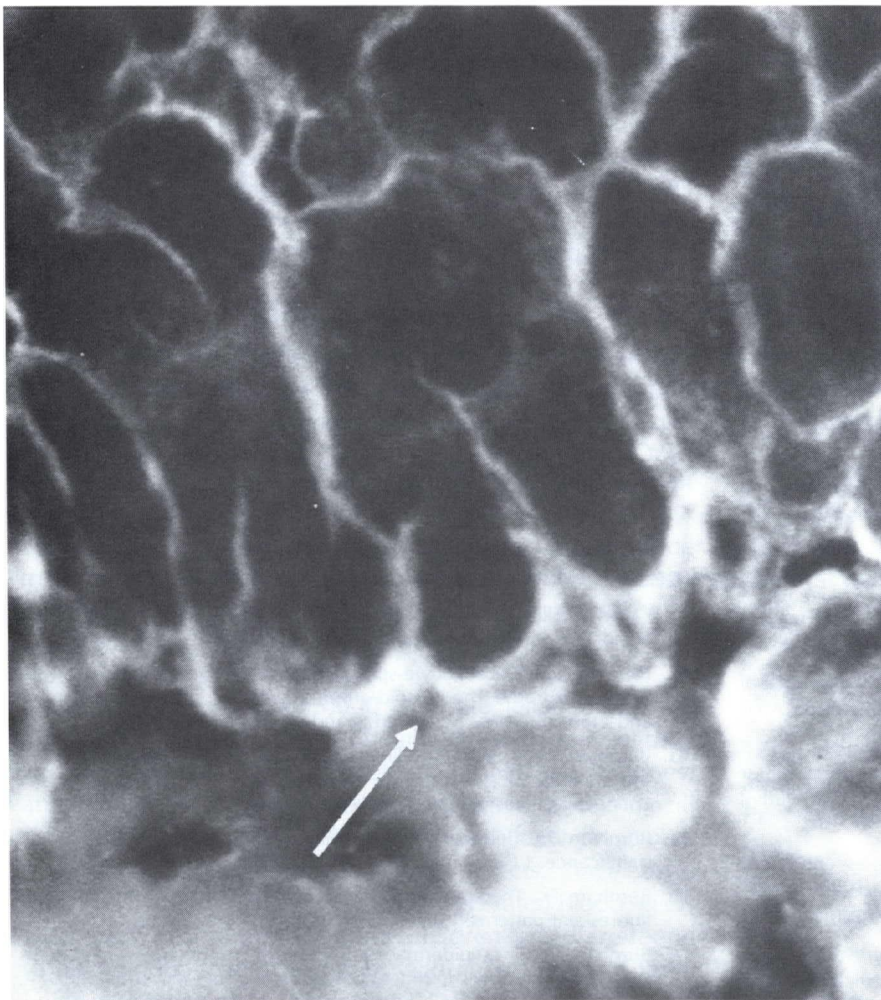
These showed the pattern similarly to its appearance on the human spleen imprints in that the positive nuclei tended to be few in number and again were randomly distributed.

#### *Human blood smears and Hep-2 cells*

These substrates have not demonstrated this pattern. Both initially frozen and fresh blood smears were negative. However, we have only recently started to test Hep-2 cells and have not had any strongly positive high-titered sera available to evaluate.

#### **Comparative morphology and titers of PLS/NM ANA pattern employing different conjugates**

No morphologic differences were found with any of the conjugates regarding this pattern. On spleen imprints, the morphology was similar with the antihuman gamma



**Fig 4**  
Peripheral nuclear ANA pattern on human normal skin section. Brightly fluorescent, mainly contiguous, thin walls depict this pattern. It can be differentiated from the PLS/NM ANA pattern on skin sections by sparser and narrower internuclear gaps and generally thinner fluorescent walls. Arrow depicts dermal-epidermal junction delineated by broad band-like wall of peripheral nuclear fluorescence of the lower parts of the basal cells. Original magnification x 320.

globulin, anti-IgG, and anti-C<sub>3</sub>, and also on the skin sections no significant morphologic differences were found between the anti-IgG and anti-C<sub>3</sub> conjugates.

The comparative titrations of the ten patients' sera on the spleen imprints with the antigamma globulin and the anti-IgG conjugates and with the anti-IgG conjugate on the skin sections showed no significant differences. This finding confirmed that the similar results from the 51 patients' sera titrated on spleen imprints and skin sections with the antigamma globulin and anti-IgG, respectively, were valid and not due to the different conjugates employed.

#### **Blocking procedures**

The PLS/NM ANA pattern was blocked both on human spleen imprints and normal human skin sections, respectively.

#### **Anti-DNA and anti-ENA results**

None of the tests of 40 patients were positive for anti-DNA or anti-ENA antibodies. Results in three patients were border

positive for IgG antisingle-stranded DNA antibodies. One of these three patients had bilateral uveitis. One of the two remaining patients had gynecomastia and abdominal pain of unknown origin; the other patient had a fever of unknown origin and myalgias that might have been of psychogenic origin. The specific diseases of the latter two patients were not diagnosed.

#### **Discussion**

The PLS/NM ANA pattern remains puzzling. We have no idea why the majority of epidermal nuclei show the pattern while usually only a few nuclei demonstrate it on human spleen imprints, and no blood smears show this pattern.

We thought that the antibody might be epidermal-nuclear preferential, but the similar titers on skin and spleen do not support this idea. Another possibility is that only nuclei in a certain stage of cell division are reactive with this antibody, but the reactivity of all the epidermal cells from the basal layer upwards is against such an explanation. The finding that the frozen blood smears were also negative rules out

the possibility that freezing is required to make the nuclei reactive to this antibody, which was a possible reason for the negative results on the unfrozen blood smears.

The lack of correlation with any specific disease is striking. Patients who have SLE do not even show this pattern without another associated ANA pattern. This is similar to our findings regarding patients who have SLE with other nonparticulate patterns (20) as discussed above. The fact that none of 100 blood donors demonstrated the pattern suggests that this antibody—although nonspecific for any diagnosis—is nevertheless produced in response to disease. The association of the pattern with poststreptococcal glomerulonephritis further demonstrates the ubiquity of disease association of this antibody.

Because of this lack of clinical significance, it is imperative that this ANA pattern be recognized and separately classified. It is therefore essential not to confuse this pattern when seen on human normal skin sections with the peripheral ANA pattern, which confirms a clinical diagnosis of SLE (1,2,9,21). The generally broader fluorescent nuclear walls and more numerous and wider internuclear gaps, than are seen with peripheral ANA, make this differentiation possible.

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