Nonhistone Nuclear Antigens Reactive with Autoantibodies. Immunofluorescent Studies of Distribution in Synchronized Cells

J. S. DENG, Y. TAKASAKI, and E. M. TAN
Division of Rheumatic Diseases, University of Colorado School of Medicine, Denver, Colorado 80262

ABSTRACT Sera from patients with certain autoimmune diseases that contained autoantibodies to nonhistone nuclear antigens were used as reagents in an indirect immunofluorescent study. The distribution of these nuclear antigens was determined in synchronized human B lymphoid cells. Autoantibodies to Sm antigen, nuclear ribonucleoprotein complex and SS-B antigen were used. Although all three nonhistone antigens appeared to show speckled nuclear staining patterns in the G0 phase, different patterns of staining were present at other periods of the cell cycle. The SS-B antigen showed a distinctly nucleolar localization during the G1/early S phase. These studies demonstrate that autoantibodies occurring in certain human diseases can be useful reagents for the immunohistological localization of nuclear macromolecules and for tracing their pathways during different phases of cell growth and differentiation.

The sera of patients with certain autoimmune diseases contain antibodies that react with nuclear components present in their native or unaltered states. Nuclear components that have been recognized to be antigenic for autoantibodies include DNA, histones, and many nonhistone proteins (1). In recent studies, an increasing number of antibodies reacting with different classes of nonhistone nuclear antigens have been recognized, and they include antibodies to Sm antigen (2, 3), nuclear ribonucleoprotein (nRNP) (3, 4), SS-B antigen (5, 6), Scl-70 (scleroderma-70) (7), and centromere antigens (8). The reasons for the formation of autoantibodies to these native nuclear components are unknown. In the clinical context, it has been shown that different disease states such as systemic lupus erythematosus, mixed connective tissue disease, Sjogren's syndrome, and scleroderma display separate profiles of antinuclear antibodies and this feature has made them useful as diagnostic markers of disease (1, 2, 4, 7, 9).

Recently, several investigators have begun to elucidate the physicochemical nature of some of these nuclear antigens. The Sm and SS-B antigens are acidic nonhistone proteins and nRNP is a complex of RNA and nonhistone protein (1, 3, 10–13). It has been shown that several species of small nuclear RNAs are components of nuclear RNP and Sm antigen (13). One of these species of small nuclear RNA, U1, has been suggested to be involved in the splicing of early transcript RNA (14).

Some autoantibodies to nonhistone nuclear antigens are present in high titers in disease sera, and are relatively nonspecific. Therefore, these sera have been used as highly specific immunohistochemical reagents to isolate antigens by immunoprecipitation (13, 14). In this study, we have selected autoantibodies to Sm antigen, nRNP, and SS-B, and used them as reagents to determine by immunofluorescence the location of the respective nuclear antigens in different phases of synchronized cells. The results show that these nonhistone components have their own specific patterns of distribution within the nucleus, and that the SS-B component is highly concentrated in the nucleolus during the late G1/early S phase of the cell cycle. These observations may be helpful in further studies to determine if these components may have differentiation or regulatory functions.

MATERIALS AND METHODS

Conditions of Cell Culture

The cell line studied was Wil-2, a line of continuously growing human diploid B lymphocytes originally obtained from the spleen of a patient with hereditary spherocytosis (15). The cells were maintained in suspension culture on a gyratory shaker. The culture medium consisted of Autopow MEM (Flow Laboratories, Inglewood, Calif.) supplemented with 2 mM glutamine, vitamins, nonessential amino acids, sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum (Flow Laboratories). The cells were seeded at a concentration of 2 x 10⁶/ml and normally subcultured every 3 d, except for the synchronization experiments described below. Cell viability was determined by 0.05% trypan blue exclusion method. For mitotic cell enumeration, the cells were treated with 0.2 μg/ml colcemid (Gibco Laboratories, Grand Island Biological

© The Rockefeller University Press · 0021-9525/81/12/0654/07 $1.00
Co., Grand Island, N. Y.) 2 h before harvesting and processed with Giemsa staining. One-ml aliquots of cell suspensions containing 2 × 10⁶ cells were pulsed with 1 μCi/ml [³H]thymidine (New England Nuclear, Boston, Mass., 6.7 mCi/mM) for 30 min. Cells were washed twice in phosphate buffered saline (PBS-0.01 M phosphate, 0.15 M NaCl, pH 7.4), collected on 0.45 μm Millipore filters (Millipore Corporation, Bedford, Mass.), extracted with 5% trichloroacetic acid, dried, and counted in a liquid TCA scintillation counter. Smears were prepared from another 1-ml aliquot of the cell suspension, fixed in methanol-acetic acid mixture (3:1) for 10 min and processed for autoradiography using Kodak NTB-2 nuclear emulsion film. The smears were developed, stained with Giemsa, labeled cells were counted, and a labeling index determined.

**Synchronization of WiL-2 Cells**

To study the distribution and migration of certain nuclear antigens in cells at different phases of the cell cycle, two methods of synchronization were used. In the first method, WiL-2 cells were synchronized by starvation or density-dependent arrest, as described by Lerner and Hodge (16). Initially, studies were made to establish the growth characteristics of our line of WiL-2 cells. WiL-2 cells were seeded from a 3-d old culture at a concentration of 2 × 10⁵/ml and the cell count, percentage of mitotic cells (mitotic index) were determined every day for 7 d without addition of fresh culture medium. By the sixth day of culture, labeling index and [³H]-thymidine uptake were <10% of maximum and the number of cells in mitosis was <0.1% of total cells. Thus, it was apparent that these cells were in a phase not related to mitosis or DNA synthesis. These results are consistent with earlier studies by Lerner and Hodge (16). For further reasons described later (see Fig. 2), the sixth day starvation cells were used as those in the G₀ phase of the cell cycle. These 6-day-old cells were used in density-dependent synchronization. Cell cultures at a density of 2 × 10⁶/ml were grown in fresh complete media. The cells were harvested every 2 h thereafter, and the phase of the cell cycle determined as described above.

In the second method, the double-thymidine block method was used as described by Galavazi and Bootsma (17). Briefly, 2-d old WiL-2 cells were treated with 2 mM thymidine (Sigma Chemical Co., St. Louis, Mo.) for 16 h. The cells were then washed in fresh media to remove thymidine and cultured again in fresh complete media. 6 h later, 2 mM thymidine was added again and the cultures were incubated for another 16 h. After the second thymidine treatment, the cells were washed and suspended in fresh complete media. Cells were harvested every 2 h thereafter, and the phase of the cell cycle determined in a manner similar to that for density-dependent arrest.

**Sera Used for Immunofluorescent Staining**

The sera used for these studies came from patients with different connective tissue diseases. Sera containing antibodies to Sm antigen were from patients with systemic lupus erythematosus, those with antibodies to nRNP from patients with mixed connective tissue disease, and sera with antibodies to SS-B antigen from patients with Sjogren's syndrome. The sera were selected from our serum bank and contained high titers of antibodies to the respective nuclear antigens. They were analyzed for antibodies to Sm and nRNP by immunodiffusion, passive hemagglutination, and counterimmunoelectrophoresis and in each of these techniques (1, 3, 18) the sera were monospecific, i.e., they contained antibody to either Sm or nRNP but not to both. Sera with antibody to SS-B were analyzed by immunofluorescence and counterimmunoelectrophoresis and in addition to the presence of antibody to SS-B, they were negative for antibodies to Sm or nRNP. Furthermore, these sera did not contain antibodies to other known nuclear antigens such as DNA, nucleohistones, ScI-70 and centromere antigens (7, 8). Nevertheless, there was a possibility that some sera might have other antinuclear antibodies that we were not detecting with the available techniques. To control for this possibility, three different sera of each antibody specificity were used to confirm that certain patterns of cell surface staining was consistently reproducible. An example of immunodiffusion analysis is shown in Fig. 1. The SS-B precipitin line intersects Sm and nRNP lines but the Sm line shows a spur over the nRNP line. The latter phenomenon should not be interpreted to denote immunological partial identity, with nRNP being antigenically deficient from Sm. The traditional interpretation of spur formation, based largely on the studies of Ouchterlony (19), were derived from interactions between antisera in center wells and antigens in peripheral wells. In Fig. 1, the placement of antigen and antisera wells is reversed. Ouchterlony has called this a Type IV reaction-inhibition pattern simulating a spur formation (19).

**FIGURE 1** Immunodiffusion study showing presence of precipitating antibodies in three representative reference sera used in synchronization and immunofluorescent studies. Sm: serum having anti-Sm activity; n-RNP: serum having anti-n-RNP activity; and SS-B: serum having anti-SS-B activity. RTE: extract of rabbit thymus containing several solubilized nuclear antigens. Each of the reference sera gave one precipitin line against RTE. The precipitation lines of Sm, SS-B, and n-RNP are immunological nonidentical (nonfusion). Whereas the line of SS-B intersects the Sm and n-RNP lines, the lines of Sm and n-RNP typically do not intersect but show spurring of Sm over n-RNP.

**FIGURE 2** WiL-2 cells synchronized by the method of density-dependent arrest. Tritiated thymidine incorporation (●●●), percentage of cells showing DNA synthesis on autoradiography (labeling index ○○○) and percentage of cells undergoing mitosis (mitotic index △△△) were determined. In the first 6-8 h of culture, >90% of cells were in a phase preceding DNA synthesis. From the eighth hour, cells began DNA synthesis and reached the maximum of the synthetic (S) phase by 16-18 h. By 22-24 h, the mitotic phase of the cell cycle was beginning, preceded by a fall in DNA synthesis. From these observations, the phases of WiL-2 cell synchronization were determined.
Immunofluorescent Staining

WiL-2 cells at Go phase or at other phases of synchronized cells were used as the substrate for reaction with the above sera. These cells were cytocentrifuged on to glass slides and fixed in acetone for 10 min at room temperature. Acetone fixation had been used previously in studies concerning Sm and nRNP and did not affect the reactivity of these nuclear antigens (3). Cytocentrifugation allowed for a flat preparation of cells so that problems with rounding or uneven thickness of cells was minimized. The cell smears were reacted with autoantibody-containing sera in the indirect immunofluorescent staining technique as described previously (3). Fluorescein isothiocyanate-labeled goat antihuman fraction II antiserum was used as the final reagent and had the following characteristics: fluorescein isothiocyanate (FITC) 132 μg/ml, protein 14 mg/ml, antibody activity 8 U/ml, and plateau end-point 1/64 dilution (20). The FITC-labeled antiserum was used at a dilution of 1/30 in PBS.

RESULTS

The results of synchronization of WiL-2 cells after density-dependent arrest are shown in Fig. 2. In the first 6 to 8 h, >90% of the cells were in a phase preceding DNA synthesis. The period of DNA synthesis began at ~10 h and reached its peak by 16 to 18 h. Thereafter, there was a fall in DNA synthesis, but significant numbers of mitotic cells did not appear until 22–24 h of culture. From these observations, it was ascertained that the period up to 8 h after release from density-dependent arrest represented the G1 phase of the cell cycle. For immunohistochemical staining, Go phase cells were taken as those immediately after release from density-dependent arrest. G1 phase cells were taken at 4 to 8 h of culture. S phase cells at 16 to 18 h (peak of DNA synthesis), and G2/M phase cells at 22 to 24 h of culture.

The observations using antibody to Sm antigen are shown in Fig. 3. In the Go and G1 phase cells, Sm nuclear antigen was distributed in a densely speckled pattern in the nucleoplasm with relatively little in the nucleolus. In the S phase cells, the nuclear staining assumed a lacework pattern, and the distinction between nucleolus and nucleoplasm was not as clear as in the Go and G1 phase cells. Sm antigen appeared to have migrated from the nuclear region into the cytoplasm in G2. In this photomicrograph, the larger cells (arrows) are cells in mitosis whereas the smaller cells were presumably in G2 phase of the cell cycle preceding mitosis. The mitotic cells were confirmed by phase contrast microscopy in this and other studies reported below.

The observations with antibody to nRNP are illustrated in Fig. 4. The staining was restricted entirely to the nucleoplasm in the Go, G1, and S phase cells. It is apparent in all these photomicrographs that the nucleolus was unstained. Further, in the G2 phase of the cell cycle, nRNP is still present in the nucleoplasm except during mitosis (arrows pointing to two cells) where nRNP antigen appears to be present in clumps in the periphery of the cell.

The observations with antibody to SS-B antigen are shown in Fig. 5. This antigen was also predominantly a nuclear antigen. An interesting finding was that, at the G1 phase, there was strong nucleolar staining which was not observed at other phases of the cell cycle. The nucleolar staining was associated with speckled nucleoplasmic staining. During S phase, the antigen was largely gone from the nucleolus and was again predominantly a nucleoplasmic antigen as in the Go phase. In

![Image](https://example.com/image1.png)

![Image](https://example.com/image2.png)

![Image](https://example.com/image3.png)

![Image](https://example.com/image4.png)

![Image](https://example.com/image5.png)

**FIGURE 3.** Fluorescent photomicrography of anti-Sm staining on synchronized WiL-2 cells at different phases of the cell cycle labeled Go, G1, S, and G2/M. In Go and G1, Sm was present in the nucleoplasm with relatively little in the nucleolus. In the S phase, it assumed a more lacework pattern, and the contrast between nucleolus and nucleoplasm was not as distinct. In the M phase, Sm antigen appeared to be aggregated in the peripheral regions of the cells, away from condensing chromosomes. In this picture (G2/M), three cells were in mitosis (arrows) but the majority were in G2. ×1200.
the $G_2$ phase, the antigen was also mainly nucleoplasmic in location. In mitotic cells (arrows), the antigen appeared to be widely distributed (Fig. 5). Because the $G_1$ phase cells were taken over a timespan from 4 to 8 h after release from density-dependent arrest, and the $S$ phase cells were taken 8 h later at the peak of DNA synthesis, it was decided to examine the cells at more closely spaced intervals to determine the precise time-points when the SS-B antigen appeared in the nucleolus. WiL-2 cells were harvested every 2 h and the percent of cells with strong nucleolar staining was determined. The peak time for the appearance of SS-B antigen in the nucleolus was between 6 and 14 h of culture, a period encompassing late $G_1$ and early $S$, the latter associated with the steep upward slope of thymidine uptake or DNA synthesis (see Fig. 2). To confirm this observation, WiL-2 cells were synchronized by double thymidine block. After release from block, the cells entered immediately into the $S$ phase. By 6 to 8 h, thymidine incorporation had peaked, and this was followed by a fall in thymidine uptake. When these cells were harvested at 2-h periods and studied with anti-SS-B sera, cells with strong nucleolar staining were shown to be present during the early $S$ phase of the cell cycle (0 to 4 h). Both these observations, therefore, confirmed that the SS-B antigen, unlike the Sm and nRNP antigens was present not only in the nucleoplasm but was highly concentrated in the nucleolus at a period consistent with late $G_1$ and early $S$ phases of the cell cycle.

A composite photomicrograph of these findings is presented in Fig. 6. Representative cells from density-dependent synchronization were used in this composite picture. Although Sm, nRNP, and SS-B antigens all displayed speckled patterns of nuclear staining in the $G_0$ phase cell, variations in the morphology of staining were apparent. There may be some cytoplasmic staining for SS-B antigen in $G_0$ cells but it was of low intensity as visualized by microscopy. In the $G_1$ phase (more correctly, late $G_1$ and early $S$, as described above) of the cell cycle, there was strong staining for SS-B antigen in the nucleolus. At peak $S$ phase, it is possible that the Sm antigen was also present in the nucleolus, because the margins between nucleolus and nucleoplasm could not be clearly separated. The different patterns of staining for the three antigens in the $M$ phase of the cell cycle is quite apparent.

**DISCUSSION**

A limitation concerning the present study is the use of whole sera as antibody reagents, although the sera were carefully selected and appeared operationally to be monospecific. The data would need to be confirmed by other investigators, using other sera or reagents such as specifically purified antibodies or monoclonal antibodies when these are available.

In an earlier study (3), WiL-2 cells were probably not synchronized as closely as in the present study and Sm antigen was thought to be both nuclear and cytoplasmic in $G_0$ phase. In the present study we have consistently observed Sm to be strictly nuclear in $G_0$, $G_1$, and $S$ phases. Earlier clinical investigations provided some information concerning the immunochernical characteristics of Sm, nRNP, and SS-B antigens. Sm antigen like many nonhistone nuclear proteins was eluted from
DEAE columns at a salt concentration of ~0.30 M NaCl. It showed anodic mobility in electrophoresis under neutral or alkaline conditions (3). It showed a rather heterogeneous molecular weight distribution, varying from 70,000 to 150,000. The antigenicity of Sm was not destroyed by digestion with DNase or RNase and was relatively resistant to trypsin digestion (3, 21). On the other hand, the antigenicity of nRNP was readily destroyed by either RNase or trypsin, but like Sm antigen was resistant to DNase digestion. This and other observations led to the conclusion that the RNP antigen was likely to be an RNA-protein complex. The difference in enzyme susceptibilities of the Sm and RNP antigens has been the basis of a number of diagnostic tests that separate the respective identities of the two antibodies. It has also been observed in physicochemical studies that Sm and nRNP antigens appear to cofractionate in many isolation procedures using DEAE column chromatography, gel filtration, and sucrose density gradient studies (3, 21, 22).

Recently, Douvas et al (10) used biochemical techniques to partially purify nRNP from rat liver nuclei, and a final isolation with antibody affinity columns. They showed that RNP antigen was composed of polypeptides of mol wt 13,000 and 30,000. No Sm antigen activity was present in this isolated material. Lerner and associates (13) used immunoprecipitation as a technique to isolate precipitating antigen from an extract of sonicated nuclei from Ehrlich ascites cells. They found that sera with either anti-Sm or anti-RNP activity both precipitated the same seven polypeptide bands identified by gel electrophoresis. These seven polypeptides varied from 12,000 to 35,000 mol wt. In addition, Lerner et al showed that anti-RNP specifically precipitated U1 species of small nuclear RNA (snRNA), whereas anti-Sm precipitated U1 snRNA as well as U2, U4, U5, and U6 snRNAs.

Antibody to the SS-B antigen has been also described as antibody to La and Ha antigens (11, 12, 23). The SS-13 and Ha antigens have been characterized as intranuclear antigens (5, 6, 11, 12), whereas, the La antigen has been described as a cytoplasmic antigen (23). Further, the SS-B and Ha antigens were reported to be protein on the basis of resistance to DNase and RNase, but susceptibility to trypsin (5, 6, 12). The La
antigen, however, was described as cytoplasmic and consisted of an RNA-protein complex because its antigenicity was destroyed by either RNase or trypsin (23).

The above discussion points to the controversial nature of the reported findings concerning the characteristics of these nuclear antigens. Some of the differences in the findings may be related to the fact that the nuclear antigens may be present in the native state as molecules associated with one another in larger-complexed particles. In a previous study, it was shown that the Sm protein had DNA-binding property and bound more tightly to single-strand DNA than to double-strand DNA (24). Although binding to RNA was not studied, it is possible that Sm protein might bind to RNA molecules in the cell nucleus, and at least some Sm molecules may be associated with RNA protein complexes. Therefore, in immunoprecipitation studies where whole cell extracts were used as the source of antigen, immunoprecipitation with either anti-Sm or anti-nRNP might isolate similar packages of RNA and proteins. This might partially explain some of the findings discussed above. The study with synchronized cells reported in this paper suggests approaches that might resolve some of these differences. For the Sm and nRNP antigens, some differences are observed in the distribution patterns of these antigens during G0, G1, and S phases of the cell cycle. A more striking difference in cellular distribution is observed during G2/M phase of cell cycle. At G2/M, the Sm antigen appears to have migrated away from the area of nuclear chromatin condensation, whereas, the RNP antigen appears to be located at the periphery of chromatin maturation and may indeed be still associated with chromatin (Figs. 3, 4, and 6). These observations clearly suggest that the Sm and nRNP antigens are involved in a dynamic fashion with intracellular growth and division. It also points to the fact that the nature of the nuclear antigens may be critically related to the phase of the cell cycle. The observations concerning the SS-B antigen are even more striking. During the late G1/early S phase, the SS-B antigen was predominantly nucleolar in location as compared to the G0 and late S phases in which it was nuclear in distribution (Figs. 5, and 6).

The current studies suggest that immunoprecipitation for the isolation and characterization of nuclear antigens should be performed in synchronized cells, because at different phases, antigens may exist as complex molecular assemblies or possibly as separate components. In addition, it is possible that information may be obtained concerning the function of some of these nuclear antigens by analysis of its association with other nuclear macromolecules during different phases of the growth cycle. Already the studies of Lerner and associates (13, 14) have suggested a splicing function for the U1 snRNA, precipitated by antibody to nRNP. Because phases of the cell cycle can be correlated with DNA, RNA, and protein synthesis or DNA replication, the use of synchronized cells might help in elucidating functional roles for these nuclear antigens.

This work was supported by National Institutes of Health Grant AM 20705 and by a Clinical Research Center grant from the Arthritis Foundation.

Received for publication 16 March 1981, and in revised form 24 July 1981.

REFERENCES

1. Tan, E. M. 1978. Autoimmunity to nuclear antigens. In: The Cell Nucleus, H. Busch, editor. Academic Press, Inc. 457-477.
2. Tan, E. M., and H. G. Kunkel. 1966. Characteristic of a soluble nuclear antigen precipitated by sera of patients with systemic lupus erythematosus. J. Exp. Med. 123:1003-1021.
3. Northway, J. D., and E. M. Tan. 1972. Differentiation of antinuclear antibodies giving speckled staining pattern in immunofluorescence. Clin. Immunol. Immunopathol. 1:140-149.
4. Sharp, G. C., W. S. Irving, E. M. Tan, R. C. Gould, and H. R. Holman. 1972. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). Am. J. Med. 52:148-159.
5. Alspaugh, M. A., and E. M. Tan. 1975. Antibodies to cellular antigens in Sjogren's syndrome. J. Clin. Invest. 55:1067-1073.
6. Alspaugh, M. A., N. Talal, and E. M. Tan. 1976. Differentiation and characterization of autoantibodies and their antigens in Sjogren's syndrome. Arthritis Rheum. 19:216-222.
7. Douvas, A. S., M. Achten, and E. M. Tan. 1979. Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. J. Biol. Chem. 254:10514-10522.
8. Moroi, Y., C. Peebles, M. J. Fritzler, J. C. Steigerwald, and E. M. Tan. 1980. Autoantibody to centromere (kinetochore) in scleroderma sera. Proc. Natl Acad. Sci. U. S. A. 77:1627-1631.
9. Alspaugh, M. A., and P. Maddison. 1979. Resolution of the identity of certain antinuclear antibody systems in systemic lupus erythematosus and Sjogren's syndrome: an interdisciplinary collaboration. Arthritis Rheum. 22:796-798.
10. Douvas, A. S., W. E. Stumph, P. Reyes, and E. M. Tan. 1979. Isolation and characterization of nuclear ribonucleicprotein complexes using human antinuclear ribonucleoprotein antibodies. J. Biol. Chem. 254:3608-3616.
11. Akizuki, M., R. Powers, Jr., and H. R. Holman. 1977. A soluble acidic protein of the cell nucleus which reacts with serum from patients with systemic lupus erythematosus and Sjogren's syndrome. J. Clin. Invest. 59:264-272.
12. Akizuki, M., J. J. Boehm-Truitt, S. S. Kasan, A. D. Steinberg, and T. M. Chused. 1977. Purification of an acidic nuclear protein antigen and demonstration of its antibodies in subsets of patients with sicca syndrome. J. Immunol. 119:952-958.
13. Lerner, M. R., and J. A. Steinz. 1979. Antibodies to small nuclear RNA complexes with proteins are produced by patients with SLE. Proc. Natl Acad. Sci. U. S. A. 76:5495-5499.
14. Lerner, M. R., J. A. Boyle, S. M. Mount, S. L. Wolff, and J. A. Steinz. 1980. Are snRNP's involved in splicing? Nature (Lond.) 283:220-224.
15. Levy, J. A., M. Violainen, and V. Defendi. 1968. Human lymphoblastoid lines from lymph nodes and spleen. Cancer. 22:517-524.
16. Lerner, R. A., and L. D. Hodge. 1971. Gene expression in synchronized lymphocyte studies on the control of synthesis of immunoglobulin polypeptide. J. Cell. Physiol. 77:262-276.
17. Galavazi, G., and D. Bootema. 1966. Synchronization of mammalian cells in vitro by inhibition of the DNA synthesis. II. Population dynamics. Exp. Cell Res. 41:638-651.
18. Nakanuma, R. M., and E. M. Tan. 1977. Recent progress in the study of autoantibodies to nuclear antigens. Hum. Pathol. 9:85-91.
19. Ouchi, O. 1964. Gel diffusion techniques. In: Immunological Methods. J. F. Ackroyd, editor. Blackwell Scientific, Oxford 46-69.
20. Shu, S. J. S, Deng, S. Vetter, and E. H. Beutner. 1975. Studies on defined immunofluorescence in clinical immunopathology. II. Relationship of F/P ratio of conjugates and staining properties in indirect immunofluorescence. Ann. V. Y. Acad. Sci. 234:559-572.
21. Mattioli, M., and M. Reichlin. 1973. Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (mR) systems in SLE sera. J. Immunol. 101:1318-1324.
22. Mattioli, M., and M. Reichlin. 1973. Characterization of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera. J. Immunol. 107:1281-1290.
23. Mattioli, M., and M. Reichlin. 1974. Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. Description of a cytoplasmic nonspecific nuclear antigen. Arthritis Rheum. 17:421-429.
24. Reyes, P., and E. M. Tan. 1977. DNA-binding properties of Sm nuclear antigen. J. Exp. Med. 145:749-754.