Brief Communication

Nucleolar Structure by Immunologic Technics

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Summary. Investigation into the nature of the nucleolus has been limited to histochemistry, electron microscopy, or to chemical analysis of nucleolar fractions of disrupted cells. In this study, fluorescent antibody technics were employed with human serum antinucleolar factors to characterize the structure of the organelle. Six immunofluorescent patterns were observed. Using selected highly specific sera with antinucleolar activity, three major antigenic structures were delineated in much the same distribution as previously described by light and electron microscopy: the pars amorpha, the nucleolema, and a distinct perinucleolar area.

Introduction. The nucleolus, an RNA-rich intranuclear organelle, can be identified in free cells or in tissue sections by a variety of optical and histochemical methods. Its structure has been described as a body of 2-10 μ in diameter surrounded by the "nucleolus-associated heterochromatin" or perichromosomal RNA. Employing electron microscopic technics, Love and Liles (4) described the nucleolus as comprising a coiled filamentous structure, the nucleolema, suspended in a structureless mass, the pars amorpha.

While the chemical and histologic features of the nucleolus have been investigated, little is known of the immunologic characteristics of this structure. Beck (1, 2) and Fennel et al. (3) reported that antibodies to nucleoli are present in the sera of some patients with rheumatic disease. In the present study a group of specific human antinucleolar antibodies were used to define the immunologic details of this interesting intranuclear organelle.

Materials and Methods. Sera were collected from patients with high titers of antinucleolar antibodies as demonstrated by a modified indirect fluorescent antibody technic (5). The immunoglobulins producing these patterns were isolated, labeled with fluorescein isothiocyanate, and examined directly or by the fluorescent antibody inhibition technic (6).

The tissue substrate was 5-μ thick frozen sections of young rat liver fixed in 40% acetone-60% alcohol. Sections were examined with a Leitz-Ortholux U-V microscope, HBO 200W OSRAM mercury burner, UGI exciter filter, an ORCIX Sine immersion darkfield condenser, and a 430K barrier filter. Photographs were taken with TRI-X 35 mm film at exposures from 30 to 180 seconds. Film was developed with Kodak D76 for maximum contrast.

Results. Eleven hundred photographs were grouped into six categories on the basis of nucleolar fluorescence distribution:

1. Nucleoli unstained in an otherwise brightly stained nucleus (Figs. 1a, b).
2. Nucleoli with one or more brightly stained, roughly spherical, large amorphous bodies (Figs. 1c, d).
3. Nucleoli similar to No. 2 but with an irregular unstained center (Figs. 1e, f).
4. An irregular body smaller than the above, surrounded by an unstained halo which, when properly sectioned, showed a coiled or lobulated structure containing smaller irregular unstained areas (Figs. 1g, h).
5. A very small intranucleolar body (Figs. 1i, j).
6. Combinations of the above.

To confirm that localization of fluorescence represented an antigen-antibody reaction, the immunoglobulins from a high titered sera which produced a pattern such as in Figs. 1g, h were isolated and labeled with fluorescein isothiocyanate (5) and examined by the inhibition technic (6). Prior or simultaneous incubation of sections with the parent unlabeled serum or immunoglobulins completely inhibited binding of the fluorescein-labeled autologous protein. Significant reduction in fluorescence was demonstrated even at a 1:100 dilution of the parent serum (Chart 1).

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Discussion. The presence of complement in the immunofluorescent precipitates was demonstrated by the substitution of extensively adsorbed fluorescein-labeled rabbit anti-human complement in the indirect fluorescent antibody technic. Patterns indistinguishable from those in Fig. 1 were produced.

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The complete inhibition of fluorescent antibody by unlabeled...
can be readily demonstrated when nucleoli are properly sectioned. Frequently a small, discrete, unstained area can be demonstrated which may represent the pars amorpha. This is selectively stained with certain sera (Figs. 1i, j). Surrounding the coiled body is a zone visualized unstained (Figs. 1g, h) and stained (Figs. 1e, f), which may represent the zone of the nucleolus-associated heterochromatin. In some preparations, all areas may be stained (Figs. 1c, d). The photographs, therefore, represent precipitation of specific human immunoglobulins and complement in areas containing antigens in relatively high concentration when compared to the rest of the cell.

It is not known whether the nucleolus and perinucleolar areas represent substances in transit in the process of immunologic alteration or actual fixed anatomic structures. However, it is clear that cells contain several antigenic materials that are distributed in a reproducible fashion within the nucleolus and its immediate environs.

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References

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