The Ultrastructural Localization of Antigens in Ehrlich Ascites Tumor Cells against Antinuclear Factors in Lupus erytematosus Sera by Peroxidase-Labelled Antibody Method

The presence of antinuclear factors in sera of patients with lupus erythematosus (SLE) has been well established[1,2]. None of these antibodies, however, exibits species or organ specificity[3]. Although studies with individual serum have revealed that a particular antibody may produce a given pattern in the nucleoplasm by the immunofluorescent antibody method[4–6], it is still difficult to detect a precise localization of the antigenic determinants in an antinuclear reaction in the nuclei.

An enzyme-labelled antibody method, which is introduced by NAKANE and PIERCE[7] has, recently, been successfully applied to detecting the intracellular antigenic substances at the ultrastructural level. Using this method, a study was made on the submicroscopic localization of antigens reacting with antinuclear factors in SLE sera.

Ehrlich ascites tumor (EAT) cells were used as nuclear antigens, since this tumor consisted of individually isolated cells and appeared to be a convenient tool for simplifying the procedures of antigen-antibody reactions, and the ultrastructures of the cells were fully described previously by several authors[8,9]. EAT cells of hyperdiploid strain were grown in the peritoneal cavity of dd mice.

Sera from 5 patients with SLE were studied. All sera showed a diffuse pattern in rat liver nuclei by the immunofluorescent method. They were used with 10 times dilutions. Normal sera from 2 healthy men served as controls.

Horseradish peroxidase (Behringer & Söhne, Mannheim) was conjugated to anti-human IgG goat antibody by using glutaraldehyde as described by AVRAMEAS[10].

EAT cells removed by i.p. puncture were fixed immediately in 4% paraformaldehyde or the mixture of 0.5% glutaraldehyde and 3% paraformaldehyde in 0.05 *M* cacodylate buffer, both adjusted to pH 7.4, for 45 min at 4 °C, and rinsed in cacodylate buffer supplemented with 0.5 *M* sucrose. The cells were then incubated either in SLE or control sera with 10 times dilution for 24 h at 4 °C, and washed thoroughly overnight with several changes of the buffer. They were, thereafter, incubated in the peroxidase-labelled anti-human IgG goat antibody for 24 h at 4 °C, and were washed thoroughly again with the buffer. After refixation in 4% glutaraldehyde for 20 min, followed by washing in the buffer overnight, the cells were stained in Graham-Karnovsky's medium[11] for peroxidase activity for 30 min at room temperature. Squashed preparations of these cells were examined with a light microscope to ascertain the stainability of the cells. These stained cells were washed 3 times in the buffer, osmificated, dehydrated, and embedded in Epon. To detect endogeneous peroxidase activity, some of these cells were stained in the same medium for peroxidase without exposure to

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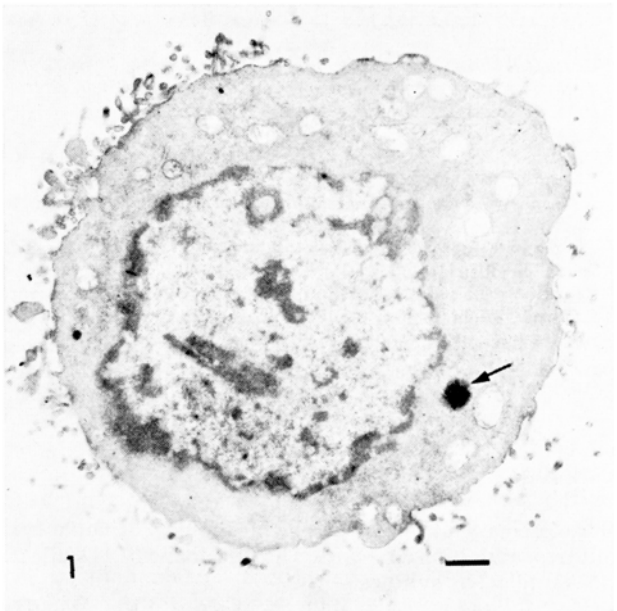

Fig. 1. An EAT cell treated with control sera, showing the electron lucent nucleus and cytoplasm. Endogeneous peroxidase activities are seen in cytoplasmic granules (arrow) and weakly in the intracristae space of mitochondria. ×6000.

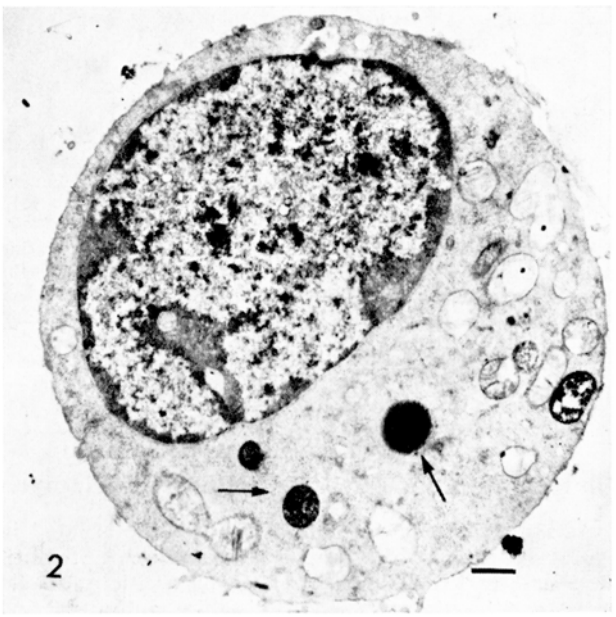

Fig. 2. An EAT cell treated with SLE sera, showing a strongly stained nuclear chromatin. In the cytoplasm, endogeneous peroxidatic activities are also seen in cytoplasmic granules, and in mitochondria. ×6000.

the antibodies. Thin sections were cut on a LKB Ultrotome, and examined in a Hitachi HU-11C type electron microscope without electron staining.

In thin sections of the EAT cells treated with SLE sera, the nuclei were stained strongly and stood out with sharp demarcation against electron lucent cytoplasm at low magnification (Figure 2). At higher magnification peroxidase activities were mainly localized in the well defined areas corresponding to perinuclear and nucleolus-associated chromatin, whereas interchromatinic zones and nucleoli remained unstained (Figure 3). Clusters of tiny granules with low electron density, corresponding probably to interchromatin granules, were observed in these interchromatinic zone. Perichromatin granules could not be identified since they were located closely to chromatin.

In the cytoplasm of SLE sera-treated cells, the intense antigenicity was occasionally found in the cisternae of the endoplasmic reticulum, though in small numbers (Figure 3).

In the cells treated with control sera, no reaction products were seen in the nuclei (Figure 1). The endogeneous peroxidase activity were seen in the homogeneous, spherical granules, approximately 0.5 μm in diameter, and in lipid droplets[12].

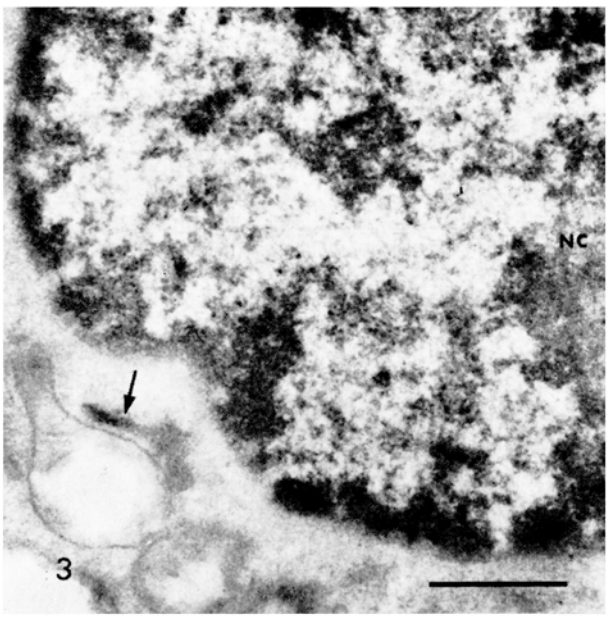

Fig. 3. Part of EAT cell treated with SLE sera, demonstrating stained chromatin and unstained interchromatinic zone. In the interchromatinic zone, fine granules of low density are visible. An arrow indicates staining of the cisternae of endoplasmic reticulum. NC, nucleolus. ×18,000.

The present results clearly show that the antigenicity associated with antinuclear factors are localized in the chromatin, and not in nucleolus or interchromatinic zone, suggesting that nuclear DNA or DNP is involved as antigens in this procedure in the light of the current concepts on the nuclear ultrastructures[13]. This is, also, compatible with the immunological assays of SLE sera by the purified antigenic substances[14–18]. In addition to the currently recommended techniques[13] including enzymatic digestion and chemical extraction, the ultrastructural immunohistochemistry employed here will be suitable for further elucidation of the topology and ultrastructure of nuclear components.

Anticytoplasmic factors in SLE sera have been described as species-nonspecific antibodies[19,20]. The antigenic constituents reacting with these anticytoplasmic antibodies have not yet been characterized. The present results may show that the intracisternal moiety of endoplasmic reticulum is one of the responsible antigens for the antibodies.

Zusammenfassung. Nach indirekter, peroxidasekonjugierter Antikörpermethode wurden elektronenmikroskopisch Ehrlich-Aszitestumorzellen geprüft und am Chromatin solcher Zellen das Antigen gegen den antinukleären Faktor in Seren einiger systemisch Lupus-erythematosus-Erkrankter gezeigt.

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On the Immunodepressive Action of Adriamycin

Adriamycin is an anthracycline antibiotic isolated in 1967 from mutant of *Streptomyces peucetius*[1,2]. Its antineoplastic action had been studied on ascitic and solid tumours induced experimentally in mice and rats[3–5] and recently in man on different neoplasms and in leukaemias of children and adults[6–10].

Recent studies[11] have demonstrated that adriamycin is able to inhibit 'blastic' transformation induced by phytohaemagglutinin (PHA) in human lymphocyte cultures and interfers with the mechanism of cellular DNA and RNA synthesis[12].

Material and methods. Male Swiss Cobs mice, weighing 20–22 g, were employed. The animals were immunized i.v. with 0.5 ml of a 2% suspension of sRBC in physiological saline. Doses and treatment schedules employed are reported in the Table. 10 animals for each experi-