PATTERN OF ANTINUCLEAR AUTOANTIBODIES IN DISEASES WITH SYSTEMIC CONNECTIVE TISSUE LESIONS

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Several types of antinuclear antibodies (ANA) were discovered by the fluorescent antibody method in diseases accompanied by systemic lesions of connective tissue and also in certain other diseases and in clinically healthy blood donors, depending on the character of fluorescence in the nuclei. Diffuse fluorescence of nuclei, diffuse fluorescence without fluorescence of the nucleoli, annular fluorescence, fluorescence in the form of granules, selective fluorescence of nucleoli, and fluorescnece in the form of long, thin, interweaving bands with simultaneous fluorescence in the region of the nuclear membrane were distinguished. The last type of ANA was observed only in various forms of lupus erythematosus, and the character of fluorescence in the nuclei differed from the "reticular" and "filamentous" types of fluorescence described previously.

KEY WORDS: *antinuclear autoantibodies; immunofluorescence; diseases of connective tissue.*

In the study of the pathogenesis of diseases with systemic involvement of the connective tissue (CT) considerable attention has been devoted to the formation of antinuclear autoantibodies (ANA). By now the method of fluorescent antibodies has led to the identification of 15 types of ANA depending on the character of the fluorescence [5]. However, since ANA are found in many other diseases, such as skin diseases [3], chronic bronchitis [2], pancreatitis [9], and burns [7], and even in clinically healthy persons [11], the further study of the various types of ANA and their specificity in diseases with systemic involvement of CT is important.

The object of this investigation was to study the pattern of ANA and their species and tissue specificity in diseases accompanied by systemic lesions of CT and to compare them with the findings in other diseases and in clinically healthy persons.

EXPERIMENTAL METHOD

ANA were investigated in five patients with systemic lupus erythematosus (SLE), 32 patients with discoid lupus erythematosus (DLE), 22 patients with infectious nonspecific polyarthritis (INP), 58 patients with rheumatic fever in the active phase, and 21 patients with the circumscribed form of scleroderma. The control group consisted of 32 patients with ischemic heart disease (IHD), 13 patients with chronic nonspecific lung diseases (CNLD) — chronic bronchitis or chronic pneumonia — 9 patients with diseases of the gastrointestinal tract (chronic gastritis, chronic cholecystitis, peptic ulcer), 23 patients with lupus vulgaris, 10 patients with bacterial eczema, and 80 clinically healthy blood donors. All patients were studied in the acute period of the disease before the beginning of treatment.

ANA were determined by the fluorescent antibodies method in the indirect variant [1]. To discover the species and tissue specificity of ANA, besides sections of rat liver, heart, and kidney tissues, other tests were carried out on sections of human and rabbit skin, esoph-

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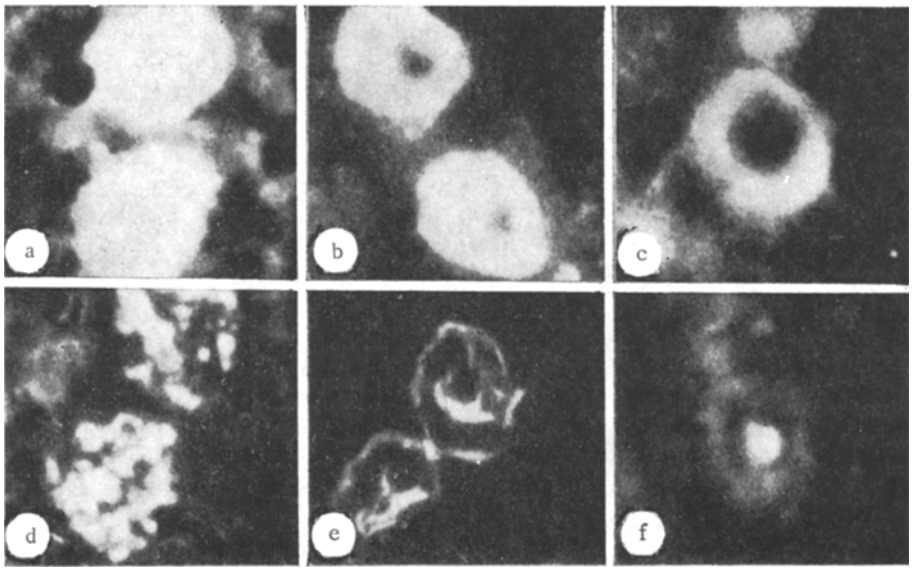

Fig. 1. Sections through rat liver. Detection of ANA in blood serum of patients by indirect fluorescence antibodies, 1500×. a) Diffuse type of fluorescence of nuclei (rheumatic fever); b) diffuse type of fluorescence of nuclei without fluorescence of nucleoli (infectious nonspecific polyarthritis); c) annular type of fluorescence of nuclei (rheumatic fever); d) granular type of fluorescence of nuclei (SLE); e) fluorescence in nuclei consisting of interwoven strand-like structures accompanied by fluorescence in region of nuclear membrane (DLE); f) selective fluorescence of nucleolus (rheumatic fever).

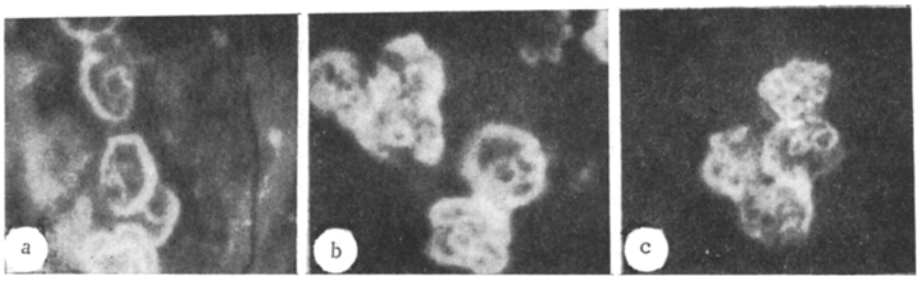

Fig. 2. Detection of ANA of the 4th type in SLE; 1500×. a) Fluorescence consisting of strand-like structures accompanied by fluorescence in the region of the nuclear membrane (section through rabbit skin); b, c) fluorescence of annular structures accompanied by fluorescence of nuclear membranes of lymphocytes (b — sections through human spleen; c — squash preparation of human spleen).

agus, skeletal and smooth muscle, and spleen, on squash preparations of human and rat spleen, and on human blood films.

EXPERIMENTAL RESULTS

ANA were discovered in all patients with SLE and in 15 of the 32 patients with DLE. ANA also were found in 30 of the 58 patients with rheumatic fever, in 10 of the 22 patients with INP, and in 2 of the 21 patients with circumscribed scleroderma.

In the control group ANA were found in 11 patients with IHD, in 4 patients with CNLD, in 1 patient with disease of the gastroentestinal tract, and in 11 patients with lupus vulgaris. No ANA were found in the 10 patients with bacterial eczema. However, ANA were observed in 2 of the 80 clinically healthy donors.

The ANA could be distinguished by the character of fluorescence in the nuclei. The following types of fluorescence of the nuclear material were observed: 1) diffuse fluorescence extending throughout the nucleus or without fluorescence of the nucleoli; 2) fluorescence of the nuclei with a marked reduction in the intensity of fluorescence in the center, which was regarded as an annular type of fluorescence; 3) fluorescence in the nucleus in the form of clearly defined granules of irregular shape and size — granular fluorescence; 4) fluorescence in the form of long thin strands, sometimes interwoven, and accompanied by fluorescence in the region of the nuclear membrane — strand-like fluorescence; 5) clearly defined fluorescence of the nucleoli against completely dark fields or superposed on weak, diffuse fluorescence in the nucleus (Fig. 1).

It was concluded from an examination of correlation between the type of ANA and the diagnosis that ANA giving the diffuse type of fluorescence was least specific. It was found equally often in rheumatic fever and INP, and also in subjects with IHD and CNLD in the control group. Diffuse fluorescence without fluorescence of the nucleoli was observed in three cases, in two patients with INP and one in the control group (IHD).

Because of the small number of observations it is impossible to draw any conclusions from these results regarding the nosological specificity of the fluorescence described as annular, or on the selective fluorescence of the nucleoli.

The granular type of fluorescence was found in the nuclei in various forms of lupus erythematosus and also in 9 of the 11 patients with lupus vulgaris.

Fluorescence in the form of interwoven strands, accompanied by fluorescence in the region of the nuclear membrane, was observed only in the various forms of lupus erythematosus.

By the use of the indirect fluorescent antibodies method to detect ANA in sections through various human, rabbit, and rat tissues no species or tissue specificity of the ANA was found. However, the clearest results were obtained with liver sections, in which all the types of fluorescence of the nuclei described above were observed. In sections through other tissues, such as kidney, heart, and skeletal and smooth muscles, granular and annular types of fluorescence of the nuclei and fluorescence of the nucleoli were not always clearly distinguishable.

The strand-like type of fluorescence accompanied by fluorescence in the region of the nuclear membrane was equally well identified in sections of various tissues (Fig. 2). Considering that many investigations of ANA have been carried out on squash preparations of the spleen [5], all the material was analyzed additionally by this method. The results agreed completely with those obtained by the other method, but whereas interwoven strand-like structures were found in the nuclei of the liver, heart, kidney, and epithelium, tiny fluorescent annular formations were observed in lymphocyte nuclei in the presence of this type of ANA (Fig. 2). In diseases with systemic lesions of CT and also in some of the observations in the control group, several different types of ANA were thus found depending on the character of their interaction with the components of the nucleus. Some of these forms are of particular interest for either they are rarely mentioned in the description of other workers or they are now described for the first time.

The granular type of fluorescence, which we observed in the various forms of lupus erythematosus and lupus vulgaris, evidently corresponds to the "nodular" types of fluorescence described previously [10] and associated with ANA to nuclear nucleoprotein.

The diffuse character of the nuclear fluorescence accompanied by total absence of fluorescence of nucleoli has not been described in diseases with systemic involvement of CT. The reason could be that sections of the liver are rarely used as the substrate for investigation by the immunofluorescence and also the infrequency of this type of ANA.

ANA giving distinct fluorescence in the nuclei in the form of interwoven strands and annular formations (in the lymphocytes), accompanied by fluorescence in the region of the nuclear membrane, deserve special attention. This type of ANA was observed only in the different forms of lupus erythematosus. The character of fluorescence in the nuclei associated with them differs from the "reticular" [10] and "filamentous" types of fluorescence [4, 6] described earlier.

There are no clear ideas at the present time on the causes and mechanisms of formation of ANA of different specificity, and their pathogenetic role in autoimmune states and diseases

with systemic involvement of CT is not always clear. For instance, lesions of the vascular system and kidneys in SLE are associated with the pathogenic action of circulating immune complexes between DNA and anti-DNA antibodies [8]. However, anti-DNA antibodies are only one type of ANA. As regards the remaining types of ANA described in the literature and in this paper, the question of their role in pathological processes connected with immune disturbances is still unexplained.

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