EXPERIMENTAL METHODS IN CLINICAL PRACTICE

Demonstration of Soluble Immune Complexes in Biopsy Tissues

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 11, pp. 545-547, November, 1995
Original article submitted January 5, 1995

The percentage of immune complexes revealed in the intercellular adhesive substance of the epidermis in acantholytic pemphigus was raised to 100% after cryostat sections of biopsy tissues were pretreated with 30-50% ethanol. Immune complexes were also found in all cases of the benign familial chronic pemphigus (Hailey-Hailey disease). It is suggested that 30-50% ethanol can gently denature the proteins and stabilize the bonds between antibody and antigen in the case of a weak affinity of antibodies, and can cause soluble immune complexes to aggregate into larger protein conglomerates, which prevents them from being flushed out of the tissues.

Key Words: *soluble immune complexes; biopsy tissues; ethanol*

The demonstration of immune complexes in tissues requires careful washing of the sections in saline [5]. However, our experience shows that some immune complexes, whose antibodies possess a weak affinity, dissociate during washing, and immunoglobulins are lost from the tissues. Moreover, soluble immune complexes may be present in tissue [4] and can be responsible for a false negative estimation.

A biopsy of skin taken from patients with certain skin diseases [2] is one of the most convenient objects for investigations in this field. It has been found that autoantibodies are directed against antigens of the intercellular adhesive substance of the stratified squamous epithelium in different forms of acantholytic pemphigus (AP) [1,5]. In many cases immunoglobulin complexes fixed in tissues are detected by the use of labeled antibodies to immunoglobulin in the intercellular spaces of the dermal epithelium and in other organs where stratified squamous epithelium occurs. But it is not unusual for the main pathogenetic factor - immune complexes in the intercellular spaces of epithelium - not to be revealed by routine immunofluorescent technique in spite of the presence of an array of clinical-laboratory signs of pemphigus such as bullae, the breakdown of the linkages between cell elements, and acantholysis [3,4]. This is observed, for instance, in Hailey-Hailey familial pemphigus (FP).

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MATERIALS AND METHODS

Serial cryostat sections of skin biopsy material from patients with AP (20 cases) and Hailey-Hailey FP (8 cases) were used for the study. Processing of cryostat sections for direct immunofluorescence was carried out as described elsewhere [2].

RESULTS

In 50% of cases of AP positive results were obtained, namely immune complexes were found in the intercellular spaces of the epidermis. In some of these cases the reactions were relatively weak. Nega-

0007-4888/95/0011-1167$12.50

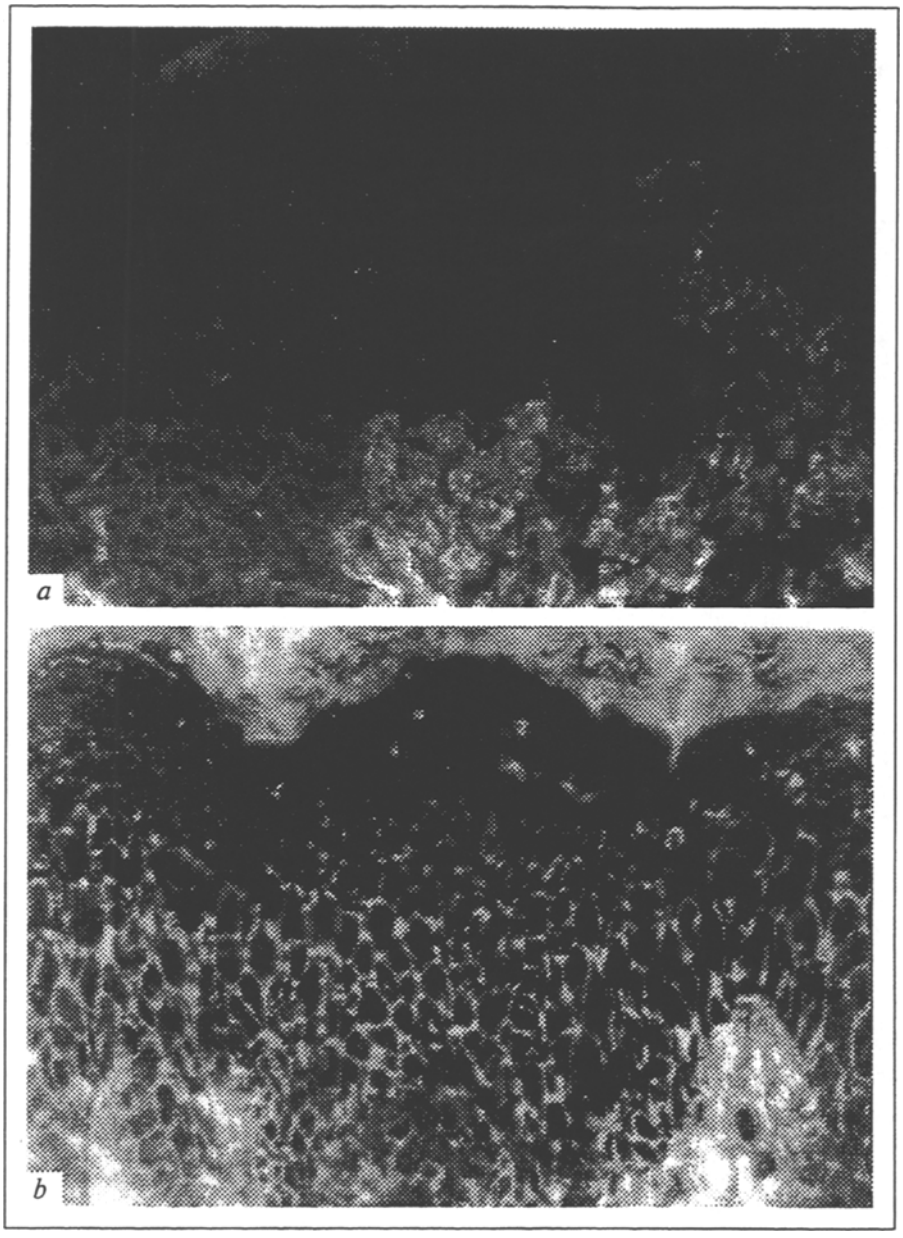

Fig. 1. Sections of skin from an FP patient. Direct immunofluorescence method. Treatment with antibodies against class G immunoglobulin. ×200. *a*) a section without additional treatment. Immune complexes not revealed; *b*) pretreatment of section with 40% ethanol. Fixed immune complexes containing immunoglobulin G in the intercellular spaces of the epidermis.

tive results were recorded in the remaining 50% of cases. In no case were immune complexes revealed in FP (Fig. 1, *a*). Sections treated with 30-50% ethanol for 1-2 min preserve the soluble immune complexes in the intercellular spaces of the epithelium in 100% of cases. Fixed immune complexes were found in the intercellular spaces of the epidermis after such treatment in all FP cases (Fig. 1, *b*). Higher concentrations of alcohol (70-100%) fix all proteins of tissue fluids in the patient's skin, masking the places where immune complexes are located. The false positive reactions which sometimes occur in dermatology in the case of immunopathological processes relating to the excretory function of the skin may be eliminated by scrupulous comparison of clinical-laboratory data.

The results obtained suggest that coagulation of aggregates of soluble immune complexes in the tissues occurs at ethanol concentrations of just 30-50% in aqueous mixtures, i.e., concentrations which are significantly lower than for fixation of soluble monomer proteins of serum and tissue fluids. In addition, under these conditions stabilization of the antibody-antigen bonds is evidently possible.

A method of demonstrating soluble immune complexes in tissues in the case of immunopathological processes in dermatology may be proposed on the basis of the findings reported here.

This study was performed as part of Prof B. A. Berenbein's investigations. We are indebted to Dr. V. A. Molochkov allowing us to carry out the study and for his useful critical comments.

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The Use of Verapamil for the Prevention and Treatment of Hypertensive Complications of Pregnancy and Its Effect on Cardiohemodynamic Indexes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 547-550, November, 1995
Original article submitted January 25, 1995

A study is performed of the efficacy of verapamil for the prevention and treatment of late toxemia of pregnancy. The preparation is shown both to produce a therapeutic effect in developed pathology and to prevent the development of severe forms of hypertensive complications in women with high-risk pregnancies.

Key Words: *verapamil; hypertensive complications of pregnancy*

At present calcium antagonists are widely used in the treatment of arterial hypertension [2,3,5]. Hypertension in pregnancy develops mainly due to a change of calcium metabolism in membranes of platelets and vascular cells [7].

Calcium ions in the circulatory bed serve as a main second messenger transmitting the signal from a surface membrane receptor into the cell. The activity of calcium channels is controlled by hormones and transmitters regulating the exchange of phosphoinositides in membranes of platelets and vascular smooth muscle cells. Activation of vascular cells by vasopressive transmitters causes an excessive influx of calcium ions into the cell, structural changes of vessels, and an increase of the total peripheral vascular resistance (TPVR). The early detection of altered vessel reactivity in women predisposed to develop hypertension in pregnancy offers the opportunity to take timely measures to forestall the development of a pathological process. One possible way of preventing hypertensive complications is to stimulate the production of endothelial relaxation factors (prostacyclin and nitric oxide), thereby restoring the balance between intracellular cAMP and cGMP and phosphoinositide signal-transduction systems [4].

We used verapamil, a blocker of calcium-ion entry into vascular cells and platelets which acts to dilate peripheral vessels and inhibit platelet aggregation, to prevent severe hypertensive complications and treat pregnant women with arterial hypertension. Isoptin-retard-240 (sustained-release verapamil) (Knoll) was used in combined therapy and did not affect the hemodynamics.

The aim of the study was to examine the cardiohemodynamic effects of verapamil in pregnant women at high risk for late tox (LT) as well as in women with hypertension and with developed LT. The body-turn test is the most practical one for identifying women at high risk of developing severe hypertensive complications of pregnancy [6].

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0007-4888/95/0011-1169$12.50