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AN EXPERIMENTAL MODEL OF PEMPHIGUS VULGARIS IN GUINEA PIGS

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The purpose of inducing experimental pemphigus vulgaris (PV) in laboratory animals is to study the mechanisms of onset of this severe autoimmune disease, which affects the skin and mucous membranes in man. It is not yet possible to cure PV, and before corticosteroids appeared the mortality was looa. Creation of a model of PV on newborn BALB/*c* mice [5] by passive transfef of the IgG fraction of serum from patients with PV (IgGPV), although confirming the pathogenetic role of autoantibodies in PV, gave no idea about the other pathogenetec factors involved in the genesis of the foremost histopathological feature of the disease, namely acantholysis (separation of cells of the stratum germinativum of the stratified squamous epithelium). This model of pemphigus likewise cannot be accepted as adequate because, with the ending of injection of pemphigus antibodies, the vesicular eruptions on the animal's skin spontaneously disappear and the mice remain alive.

In the investigation described below methods of inducing manifestations of PV in guinea pigs were screened in order to create a model of PV that corresponds to the criteria of reproduction of the autoimmune disease [12].

EXPERIMENTAL METHOD

Experiments were carried out on 146 noninbred guinea pigs weighing 150-250 g and aged 3.5-5 months. There were three series of experiments: in series I the experimental animals were given an intraperitoneal or intradermal injection of IgGPV or of blister fluid from fresh blisters on the skin of patients with PV (BFPV), into which 10^7/ml peripheral blood mononuclears from a previously untreated patient with a severe form of PV, had been injected beforehand; in series II parallel injections were given of IgGPV (intraperitoneally) and of BFPV, containing mononuclears from a patient with PV, intradermally, and in series III injection of IgGPV (intraperitoneally) was combined with intradermal injections of BFPV (containing mononuclears of a patient with PV) after heating to 56°C (30 min), or exhaustion by combined incubation with a preparation of human skin, or treatment with dexamethasone (100 μg/ml) or contrykal (10 antitrypsin units/ml), or with intradermal injections of BFPV, free from mononuclears. The IgG fraction was isolated from the blood serum of patients with PV by the sulfate-rivanol method [1, 3]. The IgG fraction thus obtained GSS "exhausted" with a human liver preparation [4] and lyophilized with a stage of juxtamural freezing. The final protein concentration was 33.2 ± 0.2 mg/ml, Control animals were given an injection of the IgG fraction of healthy human serum and BF from patients with skin burns of the II degree, containing 10^7/ml peripheral blood mononuclears from the same patients.

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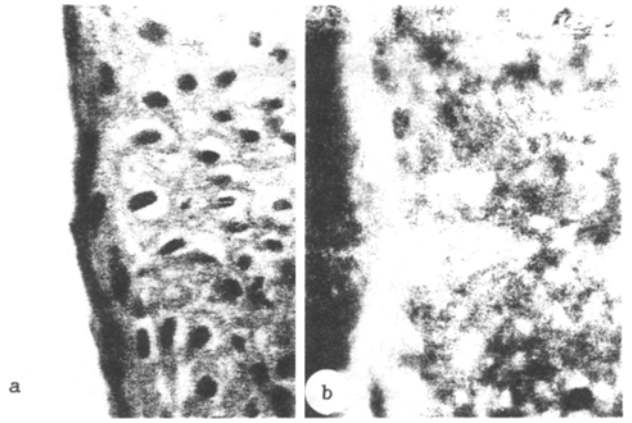

Fig. 1. Epidermie of guinea pig after intraperitoneal injections of IgGPV in total dose of 1.5 g (experiments of series I) a) vacuolar degeneration of prickle epidermocytes: cells of stratum spinosum swollen and edematous, intracellular edema with appearance of perinuclear vacuoles in cytoplasm. 1530×, stained with hematoxylin and eosin; b) intraepidermal deposits of IgGPV. 1175×, direct immunofluorescence.

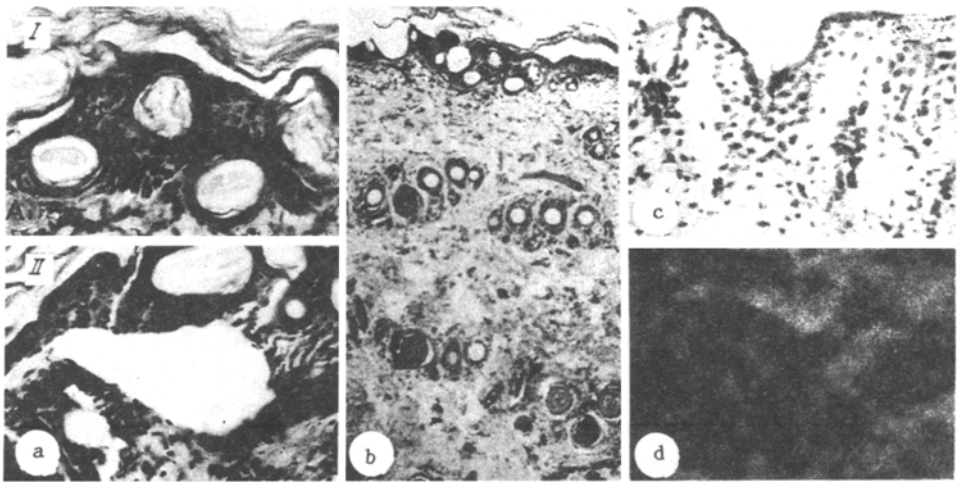

Fig. 2. Skin of guinea pig 48 h after intradermal injection of BFPV containing 10^7/ml mononuclears from patient with PV (experiments of series I). a) Balloon degeneration: blisters (I) and cavities (II) in stratum germinativum of epidermis. 1530×; a, b) stained with hematoxylin and eosin, b) dilatation of blood vessels and perivasal foci of infiltration in dermis. 170×; c) dermo-epidermal foci of infiltration consisting of eosinophils, neutrophils, and lymphocytes. 680×, stained by Romanovsky—Giemsa method; d) absence of immunofluorescence in epidermis. 1175×, direct immunofluorescence.

EXPERIMENTAL RESULTS

The experiments of series I showed that neither IgGPV nor BFPV, injected alone, caused visible changes in the skin of the experimental animals. Histological and immunofluorescence studies of skin biopsy material taken from guinea pigs receiving injections of IgGPV intraperitoneally in a daily dose of 3 mg/kg for 3-5 days revealed significant changes (Fig. 1) by comparison with those receiving other doses of IgGPV (1 and 2 mg/g daily), and also with the control animals. Vacuolar degeneration of cells of the sratum germinativum of the epidermis was found in biopsy material from visibly unchanged skin. The prickle cells appeared swollen and edematous. Their cytoplasm contained perinuclear vacuoles. Deposition of IgGPV was recorded in the lower parts of the epidermis by the direct immunofluorescence method.

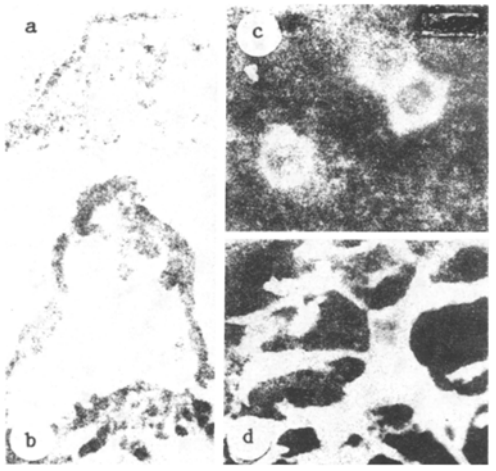

Fig. 3. Skin of guinea pig 24 h after appearance of manifestations of PV (experiments of series II). a) Acantholysis at initial stage of blister formation: intercellular edema, loss of connections between epitheliocytes, appearance of spaces and cavities in stratum spinosum of epidermis. 1530×; a, b) Stained with hematoxylin and eosin; b) intraepidermal blister: prickle cells have lost their intercellular connections, cell nucleus enlarged, some cells floating freely in cavity of blister. 1530×; c) Acantholytic cells in cavity of blister: immunofluorescence of cell membranes. 4500×; c, d) Direct immunofluorescence; d) deposits of IgGPV in intercellular space of stratum spinosum of epidermis. 4500×.

Signs of balloon degeneration, due to severe edema (Fig. 2), appeared in the stratum germinativum of the epidermis 24-48 h after intradermal injection of BFPV. Intracellular edema was combined with intercellular. This was shown by the formation of spaces and single blisters in the upper parts of the stratum germinativum of the epidermis. Dilated blood vessels and foci of perivasal infiltration were found in the dermis. Staining by the Romanovsky—Giemsa method showed that massive foci of cellular infiltration in the dermis consisted mainly of eosinophils and neutrophils, but also included lymphocytes. No immunofluorescence of the epidermis could be observed.

In the experiments of series II symptoms pathognomonic of PV appeared as a result of intraperitoneal injection of IgGPV for 2 days in a total dose of 1.5 g (6 mg/g), followed by (24 h after the end of the cycle of intraperitoneal injections of IgGPV) intradermal injection of 2.0 ml of BFPV, containing 10^7/ml of mononuclears from a patient with PV, into 10-15 sites on the shaved dorsal skin of a guinea pig. From 12 to 24 h after intradermal injection of BFPV multiple (10-27) small (pinhead size) flaccid blisters containing serous fluid appeared on the skin of the experimental animals. In response to mechanical stimulation of the skin, both affected and visibly healthy, the upper layers of the epidermis were easily separated from the lower layers (positive Nikol'skii's sign). On histologic study of skin biopsy material signs of acantholysis and extensive intraepjdermal spaces were found (Fig. 3). Early changes, preceding the appearance of blisters, could be observed in skin specimens from the periphery of the pathological foci. The initial stage of blister formation was found to be well-marked intercellular edema of the epidermis with disappearance of intercellular cross-linkages. As a result of loss of these connections between the epitheliocytes of the stratum spinosum of the epidermis, spaces were formed to begin with, followed by blisters and vesicles in a suprabasal location. Oval-shaped cells with an enlarged nucleus, i.e., acantholytic cells, were floating inside the blister. These cells were found in squash preparations from the surface of erosions formed on the skin after puncture of the blisters. Fixed IgGPV were found by direct immunofluorescence on the surface of the acantholytic cells. Immunofluorescence staining also was observed in the intercellular substance of the lower parts of the epidermis. In experimental animals with clinical manifestations of FV the titer of pemphigus antibodies was between 1/1280 and 1/5120.

In the experiments of series III, after intraperitoneal injections of IgGPV for 2 days in a total dose of 1.5 g, followed by intradermal injection of BFPV, not containing mononuclears from a patient with PV, or treated with BFPV, it was impossible to induce manifestations of pemphigus in a single guinea pig.

The cause of the appearance of acantholysis is associated with the cytotuxic action of autoantibodies of the IgG class [9], genome mutations of the epitheliocytes [2], the cytolytic effect of complement [8] and proteinases [10, 11], and also the cell-mediated cytotoxic reactions arising as a result of migration of immunocompetent cells to the dermo-epidermal junction in the skin [6, 7]. However, as our investigations showed, elimination of antibodies reacting with skin antigens, and of mononuclears from BFPV, inactivation of proteinases (contrykal) and complement (heating), and also treatment of BFPV with a glucocorticoid, prevented the appearance of pemphigus in the experimental animals. Considering that BF from burned patients had no acantholytic action, it can be tentatively suggested that the onset of acantholysis was connected with a combined effect of all the different factors given above, among which, besides pemphigus antibodies, an important role is played by mononuclears, sensitized to cell surface antigen of prickle epitheliocytes.

Considering that 10 of the 11 guinea pigs in which experimental pemphigus could be induced in the experiments of series II subsequently died, it can be concluded that the model of the disease thus created in the animal is adequate and is a true representation of the manifestations of PV: acantholysis, intraepidermal blisters, fixation of IgG in the stratum spinosum of the epidermis, and a virtually 100% lethal outcome. The suggested model of PV is readily reproducible.

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