TYPE IV COLLAGEN OF THE BASEMENT MEMBRANE OF THE SKIN EPITHELIUM IN SOME FORMS OF PATHOLOGY

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The excretory function of the skin is directly dependent on the state of the basement membrane of its epithelial structures. This function in relation to immune complexes (IC) is disturbed in several diseases [1, 4]. This disturbance is particularly marked in systemic lupus erythematosus (SLE), in which extensive deposits of granules of IC can be observed even in apparently unaffected areas of skin, in the dermo-epidermal region [4, 9]. The main components of the basement membrane of the epidermis are reticular fibers (type IV collagen) [7, 8, 13] and the ground substance, which contains glycoprotein glycoproteins [8, 10, 12]. Changes in the antigenic structure and number of compounds in the basement membrane of the epidermis are observed in a number of skin diseases and, in particular, in basal-cell carcinoma, neurofibroma [2, 6], and other diseases [11, 14].

The aim of this investigation was to compare the content of type IV collagen in the basement membrane of the epidermis in SLE and in other diseases.

EXPERIMENTAL METHOD

Biopsy material from 15 patients with SLE in the period of exacerbation of the disease was studied. The control group consisted of patients with myocardial infarction or basal-cell carcinoma and persons dying as a result of acute trauma (16 persons). Pieces of skin (3 × 3 mm) were frozen at −20 or −96°C in a mixture of dry ice and acetone. Sections 5 μ thick were cut in a cryostat (−20°C) and used either unfixed or fixed for 3-5 min in acetone or ethanol. Antiserum prepared in rabbits by the method described previously [5] was used to detect type IV collagen. Fluorescein isothiocyanate (FITC)-labeled antibodies to rabbit immunoglobulins, prepared by the usual melthod, were used in the indirect immunofluorescence test. To detect IC in the skin FITC-labeled antibodies to human IgG and antiserum to the C3 component of complement (Hylland, USA) were used. In the indirect immunofluorescence test the sections were first wetted with physicological saline, made up in phosphate buffer (PBS), pH 7.0, serum against collagen was layered above it, and the specimen placed in a humid chamber for 45 min. After washing in PBS for 10 min the sections were treated with labeled antibodies to rabbit IgG for 30 min, washed again, and mounted in 60% neutral glycerol under a coverslip. In the direct immunofluorescence test, to detect fixed immunoglobulins in the skin, the sections were treated with labeled antibodies after thorough washing for 30 min in PBS in order to remove proteins not bound with the tissues. The preparations were examined in a LYUMAM-2 luminescence microscope. They were photographed on RF-3 film under a 40× objective (water immersion) and homal 3× ocular. For histological investigation the sections were fixed in ethanol and stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

On treatment of skin sections from persons dying from acute trauma in the indirect immunofluorescence test with serum against type IV collagen, a reaction was observed in the zone of the basement membrane of the epithelium and blood vessels of the dermis (Fig. 1a). In most cases there was no reaction in the epidermis, but sometimes a few small areas with a positive

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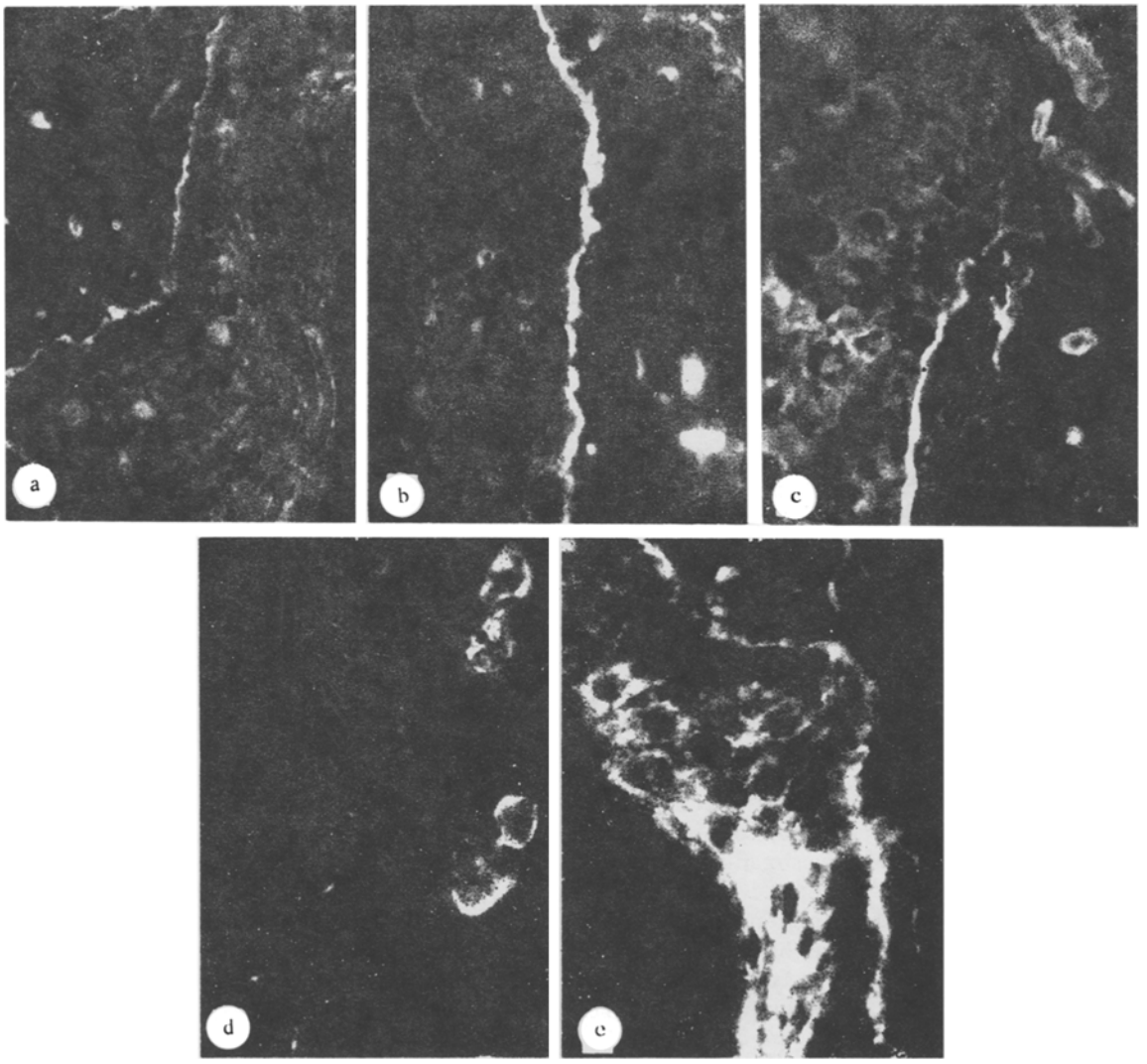

Fig. 1. Skin sections treated with antiserum to type IV collagen. a) Section through skin of clinically healthy individual: reaction in zone of basement membrane of skin epithelium and membranes of capillaries of stratum papillare of dermis; b) skin of patient dying from myocardial infarction: location of reaction the same; c) skin from patient with basal-cell carcinoma: zone of growth of tumor, area of destruction of basement membrane of epithelium as a result of mechanical action of the proliferating tumor; d) skin of patient with SLE from clinically unaffected areas: reaction in zone of basement membrane virtually absent, fluorescence preserved in zone of basement membrne of blood vessels in dermis; e) skin epithelium of patient with SLE: reaction in zone of basement membrane is unsystematic in character, much material reacting with antiserum present in the epidermis. Indirect immunofluorescence method, 120×.

reaction could be seen between the cells. A similar picture was found in skin sections from patients dying from myocardial infarction, although the distribution of collagen in the basement membrane of the epidermis was less uniform (Fig. 1b). On examination of skin sections from patients with basal-cell carcinoma, from the region of growth of the infiltrating tumor, confirmed morphologically, extensive areas of epidermis were observed in which no type IV collagen could be found. Destruction of the basement membrane evidently takes place as a result of mechanical action of the proliferating tumor. The absence of reaction in these regions was combined with discovery of collagen in neighboring intact zones and vessel walls of the stratum papillare of the dermis (Fig. 1c). Histological investigation of skin sections from patients with SLE, taken from clinically unchanged regions, showed no marked morphological disturbances, whereas in the affected zones destruction of the epidermis and of the connective-tissue structures of the skin could be seen. On treatment of the skin sections from patients with SLE,

Fig. 2. Skin sections from patient with SLE, treated with antibodies to IgG. Considerable deposits of IC visible in dermo-epidermal zone. Direct immunofluorescence method. 120×.

taken from regions with no visible changes, in 10 of 15 cases type IV collagen could not be found in the basement membrane of the epidermis (Fig. 1d). Collagen in this case was preserved completely or partially in the basement membrane of the vessels of the upper zones of the dermis. In some cases, when the reaction in the zone of the basement membrane of the epidermis was weaker, a considerable quantity of material giving a positive reaction with the antiserum was found in the form of small and large granules in the epidermis itself (Fig. 1e). On treatment of sections from the dermo-epidermal zone of the skin of these patients with antibodies to human immunoglobulin and to the C3 component of complement, extensive deposits of IC were discovered (Fig. 2). Negative correlation was found between the quantity of IC in the zone of the basement membrane of the epidermis and the intensity of the immunofluorescence reaction in sections treated with anticollagen serum. In cases when large numbers of granules of IC were present in the region of the basement membrane, no type IV collagen whatever could be found in the basement membrane or it was present in only small quantities. This can be explained by the injurious action of IC on cells synthesizing collagen in the membrane of the epidermis, as a result of which normal production of the protein is disturbed. However, the possibility cannot be ruled out that a disturbance of assembly of collagen fibers and weakening of fixation of protein in the basement membrane were taking place here. This is shown by the discovery of material giving a positive reaction with antiserum in the epithelium, i.e., the fact that collagen not utilized in the basement membrane can penetrate into the epidermis and undergo sequestration on the surface of the body. This is evidently a natural path of elimination of tissue compounds, unconnected with structures, from the body. It was shown previously that a course of hemoperfusion therapy, in conjunction with standard treatment of patients with SLE, is accompanied by disappearance of deposits of IC from the dermo-epidermal region of the skin [1, 3, 4]. Similar observations also were made in the present investigation. In 11 patients granules of IC disappeared virtually completely from the skin after treatment. The results of treatment of biopsy sections through the skin of patients with SLE, taken after treatment, with anticollagen serum, are evidence of gradual normalization of the distribution of type IV collagen in the basement membrane of the skin epithelium. In cases when collagen was not found in the membrane before treatment, a distinct reaction, although weak in the initial stages of observation (the first week) after treatment, appeared in the zone of the basement membrane. Toward the end of the period of observation (1 month) the reaction with anticollagen serum was similar to the reaction in the skin of clinically healthy individuals.

Synthesis of type IV collagen or its fixation in the basement membrane of the epidermis, disturbed during exacerbation of the disease in patients with SLE, is thus completely or partly restored as a result of treatment.

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PHOTOMODIFICATION OF HUMAN IMMUNOCOMPETENT BLOOD CELLS

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Research into the mechanisms of action of optic radiation (OR) on blood has recently assumed great practical importance [1, 8]. The study of photomodification of immunocompetent cells (lymphocytes) is particularly promising: the basic function of immunity, namely ensuring the stability of the antigenic structure of the organism, is realized through them. The immune system, as we know, is a component of the adaptation-protection mechanisms which maintain homeostasis of the human and animal body. There is information in the literature to show that UV radiation, as the biologically most active part of radiation in the optic range, induces modification of certain functions of lymphoid cells [7, 10, 12].

In the investigation described below, processes of photomodification of lymphoid cells in human blood, developing immediately after exposure to OR and also in the late stages after irradiation, were investigated by methods of spontaneous and immune rosette formation and the blast transformation test, combined with treatment with the antooxidant α-tocopherol (TP) (the results of the investigation were recorded 90 min and 3 days after irradiation).

EXPERIMENTAL METHOD

Human blood (from men aged 25-40 years) was used. Lymphocytes were isolated from stabilized blood (TsOLIPK-7B stabilizer, final concentration 1%) by centrifugation in a Ficoll-Verografin density gradient [13]. The cell suspension, in a concentration of $2 \cdot 10^6$ cells/ml, was irradiated with OR (80% of the radiant energy lay within the 280-365 nm waveband) in buffered physiological saline (0.15 M NaCl, pH 7.4), in a volume of 2 ml with continuous mixing [3]. The lethal effect was estimated in accordance with the fraction of cells stained with trypan blue (0.2% solution of the dye, from Merck, West Germany). T lymphocytes were counted as the number of cells forming "active" E rosettes (E-FRC) [15]. B-lymphocytes form identifiable EAC-rosettes (EAC-FRC) [9]. The formation of secondary lipid peroxidation (LPO) Products (malonic dialdehyde) was estimated by their reaction with 2-thiobarbituric acid (TBA-active products) [6]. To assess the functional state of the lymphocytes (ability to proliferate, blast-transformation) in the late stages after irradiation spontaneous and stimulated DNA synthesis was determined (the cells were labeled with ^{3}H-thymidine, 23-26 Ci/mmole, from Izotop, USSR) [5]. Phytohemagglutinin (PHA) was used as the mitogen (FGA-P, 8.5 μg/ml, from Difco, USA). The antioxidant TP (M. V. Lomonosov Institute of Fine Chemical Technology, Moscow), dissolved in ethanol, was added to the cell suspension or to the blood before irradiation or immediately thereafter, in a final concentration of 10^{-7} M. The ethanol concentration in the cell suspension or in the blood did not exceed 1%.

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