COMPARISON OF FREQUENCY OF DISCOVERY OF AUTOANTIBODIES TO EPIDERMAL ANTIGENS AND LEVEL OF ANTIBODIES TO GROUP A STREPTOCOCCAL POLYSACCHARIDE AND NUMBER OF SUPPRESSOR T CELLS IN GLOMERULONEPHITIS

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Glomerulonephritis (GN) is an autoimmune process which, in most cases, is linked with infection by group A streptococci [14]. Cross reactions between the polysaccharide of group A streptococcus (A-PSC) and antigens of epithelial cells of the mammalian skin and thymus have been found with the aid of polyclonal and monoclonal antibodies. Antibodies to the group-specific determinant (D) of A-PSC and antibodies to one of the rhamnose D have been shown to react with antigens of cells in the basal layer of the cutaneous epithelium (BLCE), and also with epithelium of the cortical and medullary zones of the thymus [16]. Antibodies to another rhamnose D of A-PSC react with an antigen characteristic of cells of all layers of the cutaneous epithelium and the epithelial cells of the thymus. All these antibodies are autoantibodies [6]. The epithelium of the thymus constitutes a microenvironment in which various subpopulations of T lymphocytes mature, and cells of the cortical and medullary zones belong to the endocrine epithelium of the thymus [10]. In certain autoimmune processes in man and in New Zealand (NZ) mice autoantibodies to BLCE and to the endocrine epithelium of the thymus have been found. Such autoantibodies are found extremely rarely in control sera [5]. On the basis of these findings it has been suggested that damage to the endocrine epithelium of the thymus under the influence of autoantibodies is the first stage in the onset of the autoimmune process and evidently leads to a deficiency of suppressor T cells [5]. Since A-PSC contains D common with D of different antigens of the epithelial cells of the skin and thymus, it is interesting to study autoantibodies in GN not only to BLCE, but also to the differentiated layers of the cutaneous epithelium (DLCE) Incidentally, autoantibodies to DLCE have been found in a high percentage of cases in normal blood donors [4], but they have not been studied in autoimmune conditions.

The aims of the present investigation were: to determine autoantibodies to antigens of the basal and differentiated layers of the epidermis in acute GN of varied etiology; to compare these parameters with the level of antibodies to A-PSC; to determine the number of suppressor T cells in the blood in the presence or absence of autoantibodies to BLCE.

EXPERIMENTAL METHOD

Altogether 57 sera from patients with acute GN, aged from 1 to 15 years, and 32 sera of healthy subjects of the same age (control) were tested. To differentiate between streptococcal GN and GN of different etiology [14] antibodies to O-streptolysin (obtained from the Leningrad Research Institute of Vaccines and Sera) and streptococcal hyaluronidase (from the N. F. Gamaleya Research Institute of Immunology, Epidemiology, and Microbiology, Academy of Medical Sciences of the USSR were determined by the usual methods. The level of antibodies to group-specific D of A-PSC was studied by immunodiffusion (ID) in gel with different concentrations of A-PSC in the solution [2]. An immunoenzyme (IE)

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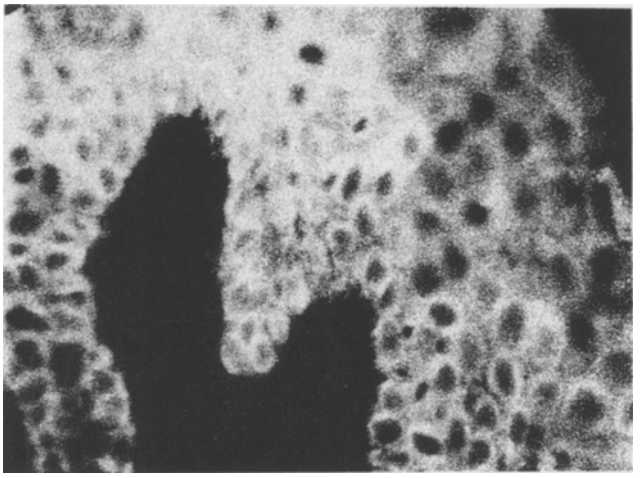

Fig. 1. Reaction of autoantibodies with BLCE and DLCE, Indirect immunofluoresce method.

method also was used, in which, besides antibodies to the group-specific D, antibodies were found to other D of A-PSC [3]. Preparations of A-PSC and streptococcal polysaccharide of the A variant (V-PSC), which is known to contain only rhamnose D, were obtained [9] from streptococci of group A (strain No. 6/49, Prague Collection) and of the A-variant (from McCarty, USA). Autoantibodies reacting with BLCE or DLCE were determined by the indirect immunofluorescence method on sections of human embryonic skin [12]. Antibodies to human IgG, labeled with fluorescein (N. F. Gamaleya Research Institute, obtained from Professor K. L. Shakhanina), were used. The reaction was read on an ML-2 luminescence microscope with 40× objective. A Homal ×3 ocular was used for photography. The reactions of the sera with the epithelium were inhibited by A-PSC or V-PSC (2 mg/ml serum), The total number of T lymphocytes and the number of suppressor T lymphocytes in the blood in GN and the control also were determined by the usual methods [11].

EXPERIMENTAL RESULTS

In the IE test antibodies to A-PSC were found in GN in 57.1 ± 7.1% of sera and antibodies to the group-specific D were found by the ID method in 30.2 ± 6.3% of cases. Sera in which antibodies to A-PSC were not found were evidently obtained from patients with GN of nonstreptococcal etiology, as was confirmed by parallel determination of antibodies to O-streptolysin and to streptococcal hyaluronidase. All sera from patients with acute GN were divided into two groups: group 1 with a high level of antibodies to A-PSC in ID (reaction with A-PSC in a concentration of 25-50 μg/ml) and in the IE test (antibody titer ≥12,800); group 2, with a lower level, and also sera not containing antibodies to A-PSC, The control sera were not divided according to their titer of antibodies to A-PSC, for a high level of antibodies in this group was found only in solitary cases (in 7.4 ± 4.9% in ID and 3.7 ± 3.7% in the IE test).

Some sera contained autoantibodies reacting only with DLCE, whereas in other sera the antibodies reacted simultaneously with BLCE and DLCE (Fig, 1). Sera reacting only with BLCE were not found. In GN, titers of autoantibodies to BLCE and DLCE were higher (1:16-1:64) than in the control sera (1:8-1:16).

Reactions with BLCE were found in sera from patients with acute GN with a high level of antibodies to A-PSC (group 1) in 53.3 ± 13.3% of cases. In group 2, with a low level of antibodies to A-PSC, or in their absence, reactions with BLCE were found in 19.0 ± 6.1%, compared with only 9.4 ± 5.1% of cases in the control sera (Fig. 2). The differences between groups 1 and 2 and also between group 1 and the control are significant ($p < 0.01$). Reactions with DLCE were found in group 1 in 60 ± 13.1% in group 2 in 54.7 ± 7.7% and in the control in 34.5 ± 8.4% of cases (Fig. 2). In group 2 and in the control, sera with autoantibodies to DLCE only predominated. In GN of nonstreptococcal etiology (without antibodies to A-PSC, O-streptolysin, and hyaluronidase) autoantibodies reacting with BLCE and DLCE were found in 23.3 ± 7.3 and in 58.8 ± 8.4% of cases respectively.

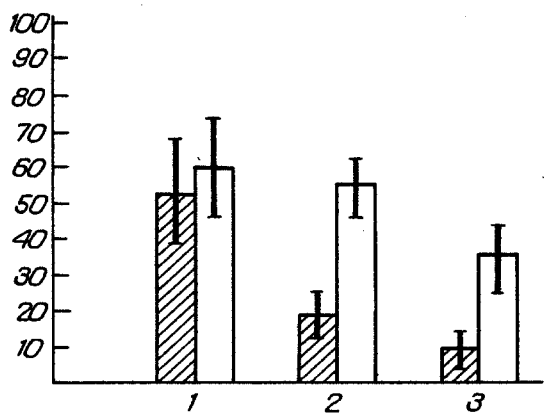

Fig. 2. Reaction of sera in GN with BLCE and DLCE depending on level of antibodies to A-PSC. 1) GN, group 1 sera (high level of antibodies to A-PSC); 2) GN, group 2 sera (low level of antibodies to A-PSC). 3) Control group. Shaded columns indicate reaction with BLCE; unshaded columns — reaction with DLCE.

According to preliminary data, in GN of streptococcal etiology of reactions with BLCE and DLCE mainly induces V-PSC rather than A-PSC. By contrast, in GN of different etiology, no appreciable inhibition of reactions with antigens of the epidermis was observed under the influence of A-PSC and also of V-PSC In the control, autoantibodies reacting with BLCE, just as in GN of streptococcal etiology, were inhibited mainly by V-PSC. It must be emphasized that all sera of the control group reacting with BLCE (9.4 ± 5.1%) contained a high level of antibodies to V-PSC.

The study of T lymphocytes in the blood of patients with GN of streptococcal etiology showed that the total number of these lymphocytes was normal Meanwhile it was shown that if antibodies to A-PSC, cross-reacting with BSCE, are present in the sera, suppressor T cells account for 12.0 ± 4.8% of the total number of T cells, whereas in the absence of these autoantibodies, they accounted for 37.2 ± 11.5%. The differences are significant ($p < 0.05$).

The results are thus evidence that acute GN, like other autoimmune processes [5, 7], is characterized by the presence of autoantibodies reacting with BLCE in a high proportion of cases. During GN of streptococcal etiology, just as in rheumatic fever [13], positive correlation is found between the titer of antibodies to A-PSC and the frequency of discovery of autoantibodies to BLCE. With which D of A-PSC these autoantibodies cross-react is a problem which requires further study. The more intensive inhibition of autoantibodies with the aid of B-PSC may perhaps depend on partial masking of the rhamnose D in A-PSC by β-N-acetylglucosamine Autoantibodies may also be directed toward different D of A-PSC depending on the stage of the disease. It has been shown in rheumatic fever that in the early stage autoantibodies mainly cross react with the group-specific D of A-PSC, but in the late stages they react with the rhamnose D [4, 13]. The decrease in the number of suppressor T cells in GN in cases when autoantibodies to BLCE are found in the sera is interesting. This is a problem which requires further study in various autoimmune processes. However, it must be pointed out that a high level of autoantibodies to BLCE and to the endocrine epithelium of the thymus is found in 100% of cases also in chronic active hepatitis B. Similar autoantibodies are virtually absent in persistent hepatitis [9]. This is in full agreement with the fact that an abrupt deficiency of suppressor T cells is found only in chronic active hepatitis B [15]. The problem of the specificity of autoantibodies to DLCE in GN and in the control requires further investigation. Autoantibodies to BLCE and to the endocrine epithelium of the thymus, compared with autoantibodies to DLCE, which react with other epithelial cells of the thymus, can evidently induce different immunoregulatory disturbances [7]. For example, in experiments on BALB/c mice the presence of antibodies to the rhamnose D of A-PSC, which react not only with BLCE but also with DLCE, correlates with the development of suppression of cellular cytotoxic reactions. Meanwhile autoantibodies to BLCE, cross-reacting with the group-specific D of A-PSC, on the other hand, prevent suppression [7]. Consequently, a further detailed study of autoantibodies to antigens of epithelium of the skin and thymus, cross reacting with different D of A-PSC, is one way of decoding the mechanisms promoting or preventing the development of autoimmune processes connected with infection by group A streptococci [7].

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NATURAL SUPPRESSORS OF HUMAN BONE MARROW TISSUE

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Suppressor cells play an essential role in the regulation of reactions of both humoral and cellular immunity. It has now been established that some of the suppressor effects are due to active production of soluble factors by the cells.

Research on experimental models [3-7] has shown that the suppressor effects of bone marrow tissue (BMT) may be determined by cells of different phenotypes [8].

Much attention is now being paid to natural suppressors (NS) of BMT [3, 11]. Their enormous role and practical importance in transplantation of BMT have been demonstrated [8, 10]. We described a preliminary densotometric analysis of bone marrow suppressor cells isolated on a stepwise Percoll density gradient [1]. As the investigation showed, the natural suppressor activity (NSA) of human BMT is a quite complex phenomenon, and it is difficult at the present time to identify the type of cells with which it is connected. It can be only tentatively suggested that these cells are suppressor cells of erythroid nature (Er-suppressors) [3].

To characterize NS further we studied the NSA of bone marrow cells from healthy blood donors, patients with solid tumors, and patients with acute leukemia in the clinical-hematologic remission (CHR) stage. The aim of the present investigation was to undertake a comparative densitometric analysis of bone marrow cells (BMC), possessing NSA, in these patients and in healthy subjects.

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