REVIEWS

Immune Cytopenias and Some Associated Problems of Immunohematology

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Immunological studies carried out in hematological patients are reviewed. Special attention is paid to hematological diseases manifested in reduced counts of formed elements of the blood and associated with the appearance of, respectively, antierythrocytic, antileukocytic, and antiplatelet autoantibodies or combinations thereof. The possibility of classifying autoantibodies on the basis of clusters of antibody reactions with target cells and some other problems are discussed.

Key Words: *anti-HLA, antigranulocytic, and antiidiotypic antibodies*

Immunohematology as a science emerged at the beginning of the twentieth century, its appearance a result of the integration of two newly burgeoning disciplines: classical immunology, studying the resistance to infectious agents, and hematology, the science studying the blood.

Investigations of blood groups and rhesus factor [33], human histocompatibility antigens [2,37], and hemolytic anemias caused by immunological factors [23] are fundamental to this science. The data accumulated to date form the basis for modern concepts on the transfusions of blood components, transplantations of allogeneic organs and tissues, and blood diseases characterized by cytopenias of autoimmune origin, etc.

The above-mentioned clinical situations are united by universal reactions of the organism to antigenically foreign formed elements of the blood, mediated by immunological antibody and cellular mechanisms. The difference is due to the existence of two types of such reactions: they may be alloimmune, that is, directed against antigens of individuals, or autoimmune, directed, as a rule, against cells of certain tissue specificity of representatives of a particular species.

This review deals mainly with auto- and alloimmune manifestations of the organism, which are still attracting the attention of scientists and physicians of many specialties. Our task is to analyze in detail some notions in this branch of science, so critical for today.

We know the dramatic story behind the development of ideas about pathogenesis of hypoplastic (aplastic) anemia (AA), a disease which is still widely debated. It develops in a healthy organism and manifests itself by a steadily declining hemopoietic function of the bone marrow (BM), differing in patients only in the rate of destruction of BM tissue and its replacement with fatty tissue. AA frequently leads to early death. There is indisputable proof that AA may be caused by exposure of the organism to some myelotoxic agents, such as benzene, gasoline, etc. However, in more than half of all cases the disease is idiopathic, and it is impossible to identify the exogenous factor responsible for its development.

In 1954, Braunsteiner hypothesized a possible autoimmune component in the development of AA [22]. Evidence corroborating this hypothesis was obtained later, when the lymphocytes of AA patients

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were discovered to inhibit the formation of erythroid and granulocytic colonies of BM cells in a culture of normal human tissues [29]. On the other hand, some doubted the validity of such an assumption, because AA patients were as a rule repeatedly treated with an erythrocytic mass and could therefore be sensitized to red cell and HLA antigens. This problem was to a certain measure solved by *in vitro* experiments with BM cells of HLA-identical sibs of AA patients.

Our investigations showed that the serum of AA patients contains autoimmune antibodies, which might also have a suppressive effect on the cells of allogeneic BM placed in diffuse chambers. A specific feature of these antibodies is that their target cells are the red cells of patients with erythremia during exacerbation [15,41]. During remission the erythrocytes of patients with erythremia lose the capacity of reacting with the serum of AA patients and regain this capacity during a relapse. The sera of patients with other hematological diseases, namely autoimmune hemolytic anemia or paroxysmal nocturnal hemoglobinuria, and of normal subjects did not react with the above cell target in any of the cases. This permitted us to conclude that autoantibodies directed against antigens of erythroid BM cells of a certain degree of immaturity play an important role in the disorders of erythropoiesis in the immune form of AA, along with the cellular factors of immunity. The immune reaction in such cases is evidently directed against BM cells possessing differentiation antigens common with those of the erythrocytes of patients with exacerbation of erythremia. Hence, attempts at using the sera of AA patients with the red cells of patients with the functional or hereditary forms of erythrocytoses appear to hold good promise. If these sera react with the red cells of erythrocytosis patients less actively than in erythremia, they may be used as a differential diagnostic reagent for discriminating between erythremia and erythrocytosis. Moreover, this property of the serum of AA patients may be used to create a diagnostic reagent in the form of monoclonal antibodies to the antigens of red cells of erythremia patients during the exacerbation stage. Just why patients with AA become sensitized against their own BM cell antigens remains unknown.

There are a number of other hematological diseases besides AA whose autoimmune nature is considered to be proven. A clear-cut organ-tissue specificity of involvement was previously proposed for the majority of them, but later this proved to be not quite true. A typical example is autoimmune hemolytic anemia (AIHA). The principal feature in the pathogenesis of this disease is increased destruction of erythrocytes because of the production of antierythrocytic antibodies followed by destruction of the formed elements of the blood loaded with autoantibodies in the blood vessels [23] or, as *in vitro* experiments demonstrated, in the spleen, evidently during passage of the blood through the malpighian bodies at the expense of B lymphocytes possessing, according to our data, receptors to the Fc-fragment of immunoglobulins [3,20].

Not only antierythrocytic, but antilymphocytic antibodies as well, were found in AIHA patients [6,17,31,41]. Autolymphocytotoxins were cold-specific and could be detected at 18-22°C. Lymphocytotoxic antibodies reacted mostly with the T population of lymphocytes. Evidently, AIHA patients have deviations in the functioning of immunoregulatory cells towards a deficit of T suppressors. Autolymphocytotoxins may suppress the function of T suppressors in AIHA patients, which normally inhibit the production of autoantibodies. Autolymphocytotoxins may be detected in lesser amounts in normal subjects as well, their incidence being appreciably higher in individuals aged over 40. Probably autolymphocytotoxins together with their target cells may be regarded as a special physiological system, mediating the immunoregulatory function [1,16]. During a cold exposure temporary immunodeficiency may occur, manifesting by reactivation of the bacterial and viral flora of the organism and by the development of a respiratory disease [7].

Immune neutropenias are similar to AA. A clearly delimited but rare group of alloimmune neutropenias deserves special attention. They are caused by transplacental penetration of maternal immune antibodies destroying fetal polymorphonuclear leukocytes during gestation. Maternal antileukocytic antibodies are as a rule leukoagglutinating and, much more rarely, granulocytotoxic. They react with polymorphonuclear leukocytes of the newborn and his or her father but not with maternal leukocytes [18,32]. Hereditary neutropenias manifesting by periodic or permanent reduction of the count of polymorphonuclear leukocytes in children are even more rare [37].

In contrast to the above-mentioned conditions, the described form of immune neutropenia is autoimmune and develops as a complication of an acute infection after intensive therapy with antibiotics, sulfa drugs, analgesics, and some other agents. Acute neutropenia may sometimes present as complete agranulocytosis. Complement-dependent cytotoxic antibodies reacting with allogeneic and autologous polymorphonuclear leukocytes are strongly expressed in such patients, particularly in children. Our studies showed that in contrast to alloimmune neutropenias of newborns, the sera of patients with autoimmune neutropenias react with the leukocytes of

the mother, father, and nonrelatives [14,41]. A high inverse correlation was noted between the titer of granulocytotoxic antibodies and the absolute count of polymorphonuclear leukocytes in the patient's blood. These data indicate that autosensitization to antibodies of polymorphonuclear leukocytes is caused not only by drug therapy, playing the role of a peculiar adjuvant, but by certain genetic prerequisites as well [14]. The fact that one of the parents of a child with immune neutropenia often had autogranulocytic antibodies is one more piece of evidence in favor of this hypothesis. Study of the capacity of sera of patients with immune neutropenia to react with T and B lymphocytes, monocytes, and platelets showed that the sera reacted with these target cells in different ways. Some sera reacted, besides with polymorphonuclear leukocytes, only with T lymphocytes, others only with B lymphocytes. Still others reacted with polymorphonuclear leukocytes and both T and B lymphocytes. None of the sera reacted with platelets. The data indicate that antigens detected by autoimmune granulocytotoxic antibodies are evidently located on cells characterized by different functional trends, similarly as the cluster differentiation (CD) antigens. Hence, it is possible that autoimmune neutropenias represent a nosological entity and are caused by autoantibodies directed against antigens of different degrees of differentiation.

Yet another autoimmune disease is idiopathic thrombocytopenic purpura (ITP), first described more than 40 years ago. A factor reducing the count of circulating platelets in normal subjects after transfusion of the plasma of ITP patients was found in ITP patients' plasma [28]. Nonetheless, immunological evidence of antiplatelet antibodies was insufficient until recently. Progress in the study of ITP was achieved only when the immunofluorescent method started to be used for detecting antibodies on the platelets [21]. However, antibodies on autologous platelets are detected by the immunofluorescent test in less than 50% of patients [10]. The potentialities of this test were largely restricted by the small number of platelets in patients' blood. Use of donor platelets in the indirect immunofluorescent test somewhat improved the detection rate of antiplatelet antibodies. We would emphasize that after contact with allogeneic platelets the sera of ITP patients were no longer capable of reacting with these formed elements of the blood [10]. Testing of eluates removed from sensitized platelets once again proved the adsorption of antiplatelet antibodies circulating in the blood on the platelets from the sera. Fluorescence of platelets sensitized with eluates differed little from the fluorescence of platelets sensitized with the serum of ITP patients. It should be noted that anti-HLA sera, too, can sensitize platelets and cause their fluorescence in the immunofluorescent test. This fact prompted the development of a system of immunological methods which help distinguish anti-HLA from autoimmune antithrombocytic antibodies in studies of allogeneic platelets. Anti-HLA antibodies are known to react with both lymphocytes and platelets. In contrast to this, antiplatelet autoantibodies causing thrombocytopenic purpura are positive only with the platelets of allogeneic donors, but not with lymphocytes. The causes of antithrombocytic sensitization are unknown in the majority of patients. Nonetheless, in some of them the thrombocytopenic episodes with autosensitization can be easily attributed to a viral or other infection which might serve as a peculiar adjuvant in sensitization and cause cross-reactions, as we showed for the major histocompatibility complex HLA-B27 and *Klebsiella* [24, 25,27] and *Yersinia* [1,12] antigens in patients with Reiter's syndrome and the peripheral form of Bechterew's disease.

Notable progress has been achieved in the study of the role of the major histocompatibility antigens in transfusiology. At first it was not clear why transfusions of allogeneic platelets did not exert a hemostatic effect and did not affect the count of platelets circulating in the recipient.

Refraction to transfusion of allogeneic platelets was explained by some immunological factors, such as hyperemia, splenomegalia, etc. Later some scientists, including the author, demonstrated that anti-HLA antibodies are the principal factor responsible for the development of nonhemolytic transfusion reactions and accelerated elimination of transfused allogeneic platelets in patients with lymphoproliferative diseases and AA repeatedly transfused allogeneic platelets [19]. The higher the level of anti-HLA antibodies, the lower the increment in the count of platelets due to transfusion; frequently there was no increment in the count of platelets at all. In none of such cases was the refraction to transfused platelets caused by antiplatelet alloimmune antibodies, possibly on account of rare sensitization to platelet antigens Zw^a and Zw^b.

The dose of histocompatibility antigens plays an important role in the immunological relationships of the recipient organism and foreign cells. Experiments demonstrated that transplantation of allogeneic tissue induces an immune reaction in the recipient, followed by graft rejection [5,35,36]. The intensity of the recipient immune response may be regulated by changing the quantity of allogeneic material transplanted to the host. Experiments on rabbits and rats demonstrated that the life span of skin allotransplants shortened as the size of the skin flap increased [34, 36]. In these experiments the transplanted skin flaps varied in size from several square millimeters to 1

cm^2. Further studies revealed that this regularity was true only up to a certain point. Increase of the size of transplanted allogeneic skin flap required a longer period (several months) for its engraftment [4, 5,40]. This qualitative alteration of the recipient reaction to allogeneic transplant is observed with grafts as large as 1/3 to 1/2 of the total area of animal body surface. The totality of histocompatibility antigens entering the recipient organism from the transplanted massive skin flap led to depression of the immunological histocompatibility reaction. This was expressed as a reduced level of antibody production and round-cell infiltration of the graft [40]. The detected regularity of specific suppression of the immunological reaction of tissue incompatibility is confirmed clinically during allogeneic transplantations of large organs, such as the liver, etc., during which the incompatibility reactions are less expressed.

The above data suggest that the best results of allotransplantation can be expected if donor and recipient HLA completely coincide. It was for this reason that for more than 20 years, allogeneic BM was transplanted to patients with acute leukemia from HLA-identical sibs, who are estimated to occur among the patients' brothers and sisters in 25% of cases. However, examinations of such families revealed that there was no equality in the distribution of parent haplotypes among sibs. As a result of the irregular distribution of parental HLA haplotypes among the children, the number of sibs identical to the patients in terms of the HLA complex was appreciably increased in such families [11]. The new data shed new light on the problem of close-relative donorship of BM. Although it is relatively easy to select an HLA-identical donor of BM among the sibs in families with many children, it is doubtful that the sibs identical to the patient in both parental HLA haplotypes will be the best donors of BM. Our estimates indicate that in almost half of the cases the identity is the result of genetic disorders in gamete formation [11]. Therefore, an unrelated donor of BM, similar to the patient in HLA phenotype, is biologically preferable.

Great efforts are made to reduce the allo- or autosensitization by lowering the level of circulating antibodies. This is true for both transfusiology and organ and tissue transplantation. The method of massive repeated plasmaphereses is widely used for this purpose [13]. We think, however, that the removal of insufficient volumes of plasma may result in a boosting of antibody production (the ricochet syndrome) and in an appreciable increase of the titer of anti-HLA antibodies [9].

Use of immunoglobulin preparations binding allo- or autoimmune antibodies after the antiidiotypic exposure type appears to be a promising trend [39]. We discovered a fundamental possibility of producing antiidiotypic anti-HLA antibodies [8]. Studies are in progress, aimed at obtaining immunoglobulins from serum with HLA antibodies; these immunoglobulins are intended to contain, besides anti-HLA, antiidiotypic antibodies as well [13]. Special attention should be paid here to studies of the capacity of immunoglobulins for intravenous injections to bind anti-HLA antibodies. Such immunoglobulin has been effectively used in transplantation of allogeneic kidney and BM [38], therapy of Werlhof's disease, etc.

Hence, these data indicate that an autoimmune reaction to antigens of a certain formed element of the blood underlies numerous hematological diseases involving a deficit of a particular cellular component in the peripheral blood. Such immunologic reactions are to a great extent antibody reactions. The immune form of AA and neutropenia may be associated with the production of antibodies to differentiation antigens of the erythroid and myeloid cell elements. Antibodies are particularly diverse in autoimmune neutropenia. Differences in the autogranulocytotoxic antibodies apparently determine the degreee of involvement of the immunological systems of the patient's organism.

The role of anti-HLA antibodies in transfusiology has appreciably increased in recent years because of a wider use of allogeneic platelets as transfusion medium. They have been shown to play a major role in the development of refraction to transfused platelets.

A certain similarity has been revealed between some HLA and bacterial agents causing antigenic mimicry of bacteria and responsible for the specificities of HLA distribution in some diseases.

Use of immunoglobulins with antiidiotypic antibodies offers promise for reducing the activity of anti-HLA. *In vitro* use of these immunoglobulins helps lower the activity of anti-HLA sera. The therapeutic effect of intravenous γ-globulin in Werlhof's disease, autoimmune hemolytic anemia, the inhibitory form of hemophilia, and some other diseases is apparently due to interaction between the antiidiotype and auto- or alloantibodies.

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