MICROBIOLOGY AND IMMUNOLOGY

Autoantibodies Similar to Antidifferentiation Ones in Immune Neutropenias

E. A. Zotikov, M. N. Vasil'eva, L. P. Poreshina, R. M. Kut'ina, N. A. Finogenova, E. A. Mamedova, and T. V. Polovtseva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 124, No. 4, pp. 427-430, April, 1998
Original article submitted March 5, 1997

Two groups were distinguished among patients with autoimmune neutropenia with manifest antigranulocytic antibodies. One group consisted of patients whose sera reacted with all forms of leukocytes (granulocytes, monocytes, T and B lymphocytes), like CD45 monoclonal antibodies. Sera of patients from the other group reacted only with polymorphonuclear leukocytes and monocytes, similarly as CD13 monoclonal antibodies. Immunologically distinguished types of neutropenia were characterized by a typical chronic or acute clinical course.

Key Words: *cluster of differentiation antigens; antigranulocytic antibodies; immune neutropenias*

Immune neutropenias are little studied serious diseases. Antineutrophilic antibodies detected in patients with neutropenia have not been characterized in sufficient detail [4,5,10]. Recent studies with monoclonal antibodies (Table 1) open a novel approach to the investigation of the disease [1].

MATERIALS AND METHODS

Sera of 15 patients with immune neutropenia aged 1-61 years were examined. Clinically the disease was characterized by acute or chronic neutropenia with or without concomitant bacterial or viral infections or local inflammations. Sera of all patients contained granulocytotoxic antibodies. In the majority of sera these antibodies were detected in titers 1:16-1:128. In three patients the titer of antibodies was no higher than 1:8.

Complement-dependent cytotoxic test was the principal method of investigation [8,10]. Serum (1 μl) was pipetted into 60-well plates under Vaseline oil, an equal volume of suspension with cell concentration of 3000-4000 cells/mm^3 was added, and the mixture was incubated for 1 h with polymorphonuclear leukocytes and monocytes at 4°C and with B and T lymphocytes at 18°C. Then 5 μl complement (fresh or lyophilized rabbit serum) was added into each well.

The plates were incubated for 1.5 h at ambient temperature, after which the contents of the wells was then discarded and 1 μl 1% Trypan Blue was added in each well. After 5 min, the reaction was assessed by counting dead and live cells. The result was considered positive if at least 50% cells were dead (2 points); no more than 10% cells were dead in control wells with nonimmune AB(IV) serum (0 points).

Department of Immunohematology, Hematology Research Center, Russian Academy of Medical Sciences; Department of Follow-Up and Rehabilitation, Institute of Pediatric Hematology, Ministry of Health of Russia, Moscow

0007-4888/98/0004-0377$20.00

Table 1. Reaction of Monoclonal Antibodies with Formed Elements of the Blood

Differentiation antigen	Distribution of differentiation antigens in formed elements of the blood (in order of intensity of expression)
CD2	T lymphocytes, thymocytes
CD3	Mature T lymphocytes
CD5	T and B lymphocytes
CD7	T lymphocytes, NK cells, thymocytes, T lymphocytes of patients with acute lympholeukemia
CD8	T suppressors, thymocytes
CD9	B lymphocyte, monocyte, and platelet precursors
CD10	Lymphoblasts, granulocytes
CD11	Granulocytes, monocytes, NK cells
CD13	Granulocytes, monocytes
CD14	Monocytes, tissue macrophages
CD15	Peripheral blood granulocytes
CD16	NK cells, granulocytes, macrophages
CD17	Granulocytes, monocytes, platelets
CD19	All B cells
CD22	Mature B lymphocytes
CD24	B cells, granulocytes
CD30	Activated T and B lymphocytes
CD31	Monocytes and macrophages
CD32	Monocytes, granulocytes, platelets, B lymphocytes
CD34	Endothelial cells
CD36	Monocytes, platelets
CD39	B lymphocytes, macrophages, blood vessel endothelial cells
CD41	Platelets
CD44	B and T lymphocytes, monocytes, granulocytes, erythrocytes, Kupffer cells
CD45	All leukocytes (B and T lymphocytes, granulocytes, monocytes)
CD61	Platelets, megakaryocytes

Granulocytes were obtained by 20-min centrifugation of defibrinated or heparin-treated blood at 400*g* in a double density gradient: upper with specific density 1.080 and lower 1.120 [4,5].

The mixture of T and B lymphocytes was prepared by centrifugation of fibrin-free or heparin-treated blood in 1.080 density gradient for 40 min at 400*g*. T lymphocytes were isolated by flotation at 37°C on Nylon fibers detaining B lymphocytes. B lymphocytes were isolated by subsequent washing of Nylon fibers [7]. Monocytes were isolated from heparin-treated blood as described elsewhere [3] or by culturing the blood of patients with monocytic leukemia. Macrophages were obtained from bronchial lavage from patients with silicosis or other lung diseases, including cancer. Bronchial lavage was centrifuged in a double density gradient for 20 min at 400*g*. Macrophages were found mainly in the upper part of the gradient and at the interface between the upper and lower parts. The resultant cells (polymorphonuclear leukocytes, macrophages, and monocytes) were washed three times in Hanks' medium, treated with 0.02% iodacetamide, and a suspension containing 2000-3000 cells/mm^3 was used in the cytotoxic test.

Serum activity with platelets was assessed by the immunofluorescence test [2,6]. Platelets were isolated by 20-min centrifugation (500*g*) of the blood collected in 5% EDTA.

RESULTS

The patients were divided in two groups (Table 2). Group 1 consisted of 9 patients (Nos. 1-9), 4 of them children aged 4-12 years and 5 adults aged 33-50 years. Sera of these patients reacted with polymorphonuclear leukocytes, B and T lymphocytes, monocytes (macrophages), but not with platelets or erythrocytes. The activity with granulocytes was higher than that with T and B lymphocytes. With macrophages, serum activity was sufficiently high. The titer of antibodies corresponded to 1:32 dilution. The majority of patients in group 1 had chronic immune neutropenia, one patient had aplastic anemia and another immunodeficiency.

The disease ran a protracted many-year sluggish course with periods of decreased leukocyte count, which was associated with upper respiratory diseases, bronchitis, sinusitis, periodontitis, and other manifestations of immune failure.

Group 2 consisted of 6 patients (Nos. 10-15). The majority were children aged 1-5 years. Two other patients were adults aged 34 and 61 years. Their sera reacted with polymorphonuclear leukocytes and monocytes (macrophages) mainly in 1:32 titers but not with B and T lymphocytes, platelets, and erythrocytes. Clinical picture of the disease in group 2 was different. It was characterized by acute neutropenia, sometimes complete agranulocytosis and fever. As a rule, the disease started as an acute infection with an immune antigranulocytic component.

Our studies demonstrated several forms of immune neutropenias with typical autoantibodies. Sera of group 1 patients reacted similarly as monoclonal antibodies to CD45 antigen (Table 2). CD45-like autoantibodies are less aggressive toward neutrophils.

Table 2. Activity of Sera from Patients with Immune Neutropenias

Patient No.	Age, years	Diagnosis	Activity of antibodies with formed elements of the blood							CD-like specificity
			B lymphocytes	T lymphocytes	phagocytic cells			platelets	erythrocytes	
					granulocytes	monocytes	macrophages			
1	50	Chronic immune neutropenia	1:8	1:16	1:128		1:32	—	—	CD45
2	50	the same	1:16	1:4	1:128		1:32	—	—	CD45
3	40	Aplastic anemia	1:8		1:16			—	—	CD45
4	48	Immune neutropenia	1:8	1:16	1:64	+4	1:8	—	—	CD45
5	33	Cyclic neutropenia	1:2	1:2	1:32	+4	1:8	—	—	CD45
6	4.5	Immune neutropenia	1:64	1:64	1:128			—	—	CD45
7	8	the same	1:8	1:8	1:64	+4		—	—	CD45
8	10	Chronic immune neutropenia	1:32	1:32	1:128	+3		—	—	CD45
9	12	Immunodeficiency	1:16	1:16	1:32		1:32	—	—	CD45
10	4.5	Immune neutropenia	—	—	1:32	+4	1:8	—	—	CD13
11	7	the same	—	—	1:8	+2	1:8	—	—	CD13
12	4	the same	—	—	1:4	+4	1:16	—	—	CD13
13	1.1	the same	—	—	1:2		1:4	—	—	CD13
14	34	the same	—	—	1:32		1:16	—	—	CD13
15	61	Haptene agranulocytosis	—	—	1:32	+4	1:4	—	—	CD13

Note. Negative result (-); 50% (+2), 75% (+3), and 100% (+4) stained (dead) cells; — not determined.

This may be explained by the fact that antibodies are directed toward the differentiation antigen which is present on all leukocytes (neutrophils, monocytes, macrophages, T and B lymphocytes). In group 2, autoantibodies were similar to monoclonal antibodies to CD13 antigen (Table 1) and reacted with only two forms of leukocytes, neutrophils and monocytes (macrophages). The damage inflicted by them to neutrophils was more severe. The disease ran an acute course.

Our data are supported by the recent findings that autoantibodies detected in a patient with immune thrombocytopenic purpura were similar to monoclonal antibodies to CD9 differentiation cluster antigen [9].

Thus, our results suggest that chronic and acute neutropenias are characterized by production of two types of autoantibodies similar to anti-CD45 and anti-CD13 antibodies, respectively.

REFERENCES

1. A. Yu. Baryshnikov, Z. G. Kadagidze, A. A. Makhonova, and N. N. Tupitsyn, *Immunological Phenotype of a Leukemic Cell* [in Russian], Moscow (1989).
2. E. A. Zotikov, N. M. Katadzhan, and L. G. Kovaleva, *Gematol. Transfuziol.*, No. 12, 7-10 (1986).
3. S. K. Ackerman and S. D. Douglas, *J. Immunol.*, **120**, 1372-1374 (1978).
4. J. W. Blaschke, C. D. Severson, and J. S. Thompson, in: *Manual of Tissue Typing Technique*, Bethesda (1976), pp. 86-89.
5. J. W. Blaschke and R. V. Sozensen, *Transplant. Proc.*, **9**, 1899-1906 (1977).
6. A. E. von Borne, F. M. Helmerhorst, E. F. von Leewen, *et al., Brit. J. Haematol.*, **45**, 319-327 (1980).
7. R. P. Lowry, A. E. Person, J. E. Goguen, *et al., Transplant. Proc.*, **10**, 883-887 (1978).
8. P. I. Terasaki, D. Bernoco, M. S. Park, *et al., Am. J. Clin. Pathol.*, **69**, 103-106 (1978).
9. M. Yanabu, S. Nomura, T. Fukuroi, *et al., Brit. J. Haematol.*, **84**, 694-701 (1993).
10. E. A. Zotikov, L. P. Poreshina, and A. P. Shpakova, in: *Hematology and Transfusiology*, Moscow (1986), pp. 49-60.