Direct Antiglobulin Test-Negative Autoimmune Hemolytic Anemia

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Warm autoimmune hemolytic anemia (AIHA) is a rare type of anemia caused by the reaction of auto-antibodies with red blood cell (RBC) surface antigens at body temperature, leading to the hemolysis of RBCs [1]. The direct antiglobulin test (DAT) (also known as the direct Coombs test) was first described by Coombs, Mourant, and Race in 1945 [2] and is still an essential assay for the diagnosis of AIHA. The DAT can detect RBC-bound IgG and complement (C3), which opsonize RBCs. However, 5–10% of patients with AIHA are DAT negative [3]. The principal reasons for DAT-negative AIHA are: (a) RBC-bound IgG being below the threshold of detection by standard methods; (b) low affinity of IgG; and (c) RBC-bound IgA or rarely IgM [4]. To achieve greater sensitivity, alternative DAT methods have been developed, such as the micro-column (gel), solid-phase, polybrene, and flow cytometry methods, as well as enzyme-linked antiglobulin, immunoradiometric, and mitogen-stimulated tests [4]. Moreover, because washing RBCs with room temperature saline causes the low-affinity antibodies to be eluted from RBCs, washing RBCs with cold saline or with low ionic strength saline (LISS) may be effective in detecting low-affinity antibodies.

Because the prevalence of DAT-negative AIHA is low, the relative proportions of each of the aforementioned

reasons for DAT-negative AIHA have not been clarified. Moreover, a diagnostic algorithm for DAT-negative AIHA has not yet been established.

In this issue of the journal, Kamesaki and Kajii [5] report a detailed laboratory analysis of patients with DAT-negative AIHA and propose a classification of DAT-negative AIHA using a combination of several DAT methods. They studied patients with undiagnosed hemolytic anemia who showed negative results using the routine tube method of DAT (TM-DAT), who were further investigated with anti-IgA and anti-IgM sera and the column agglutination method of DAT (CM-DAT), and with immunoradiometric quantitation of RBC-bound IgG. According to the results, they proposed a classification of DAT-negative AIHA into 5 groups as follows: (1) positive CM-DAT after washing RBCs with phosphate-buffered saline (PBS) and RBC-bound IgG in the immunoradiometric assay over the cutoff value (the cutoff value of RBC-bound IgG is 78.5 IgG molecules/RBC [6]); (2) negative CM-DAT and RBC-bound IgG in the immunoradiometric assay over the cutoff value; (3) negative CM-DAT after washing RBCs with PBS, but positive TM- or CM-DAT after washing RBCs with LISS and RBC-bound IgG in the immunoradiometric assay under the cutoff value; (4) negative CM-DAT after washing RBCs with PBS, but positive TM- or

CM-DAT after washing RBCs with LISS and RBC-bound IgG in the immunoradiometric assay over the cutoff value; and (5) positive TM-DAT with anti-IgA or anti-IgM sera. The estimated relative fractions of patients with DAT-negative AIHA resulting from RBC-bound IgA or IgM, low-affinity IgG autoantibodies, and RBC-bound IgG autoantibodies under the threshold level of positive DAT were approximately 5, 15, and 80%, respectively.

The study by Kamesaki and Kajii [5] indicated that most cases of DAT-negative AIHA were due to RBC-bound IgG being below the threshold of detection by standard methods, followed by cases of low-affinity IgG and RBC-bound IgA or IgM. When AIHA patients are TM-DAT negative, the next step may be to evaluate RBC-

bound IgG by an immunoradiometric method, then with low-affinity IgG autoantibodies or with anti-IgA or IgM sera. However, there are many other DAT methods, and it is a challenge to choose which combination of methods to use. Moreover, we should keep in mind that these tests for DAT-negative AIHA have a low predictive value, and thus the results must be interpreted based on the clinical status of the patients.

Nevertheless, Kamesaki and Kajii's [5] work in this issue casts a new light on DAT-negative AIHA. This classification of DAT-negative AIHA can reduce misdiagnosis rates and may be useful in predicting the prognosis of each DAT-negative AIHA type. Further development in this field will be expected.

References

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