When Is a Heterophile Antibody Not a Heterophile Antibody? When It Is an Antibody against a Specific Immunogen

Heterophile antibodies are antibodies produced against poorly defined antigens. These are generally weak antibodies with multispecific activities. Human anti-animal antibodies that develop as a result of treatments with animal immunoglobulins are antibodies with strong avidities, produced against well-defined antigens. Although heterophile antibodies and human anti-animal antibodies interfere with immunological assays by similar mechanisms, modes for identifying the sources of the antibodies and for circumventing or retarding the interference may differ. Unfortunately, there has not been a well-organized attempt to encourage correct definition of these antibodies. This problem of inexact definition is highlighted by recent articles in this Journal. In the present discussion, we examine the history leading to this problem and discuss the origins and the reasons that the nature of the antibody is important for rectifying the problem. We propose a simple nomenclature for general usage that should appropriately characterize these antibodies in most cases.

We enjoyed reading the interesting article entitled, “False increase in C-reactive protein attributable to heterophilic antibodies in two renal transplant patients treated with rabbit antilymphocyte globulin”, by Benoist et al. (1) in this Journal. The authors showed that, after treatment with rabbit antilymphocyte globulin, human anti-rabbit antibodies interfered with nephelometric/turbidimetric assays for C-reactive protein. The authors point out that this type of endogenous antibody interference with turbidimetric type assays is unusual. We do not generally think of heterophile interference as affecting turbidimetric assays. In fact, pretreatment with 40 g/L of polyethylene glycol, which reduces the IgM concentration ~80% but rarely precipitates the analyte, previously has been shown to eliminate heterophile interferences in C-reactive protein nephelometric assays (2). The article presents a question that we believe is noteworthy. Were these truly heterophile antibodies? That is to say, should specific human anti-animal (immunoglobulins) antibodies (HAAAs) be referred to as specific HAAAs rather than heterophile antibodies whenever possible?

Historically, heterophile antibodies have been sheep cell agglutinins associated with mononucleosis. These antibodies are developed against poorly defined immunogens. “Hetero” and “phile” are from the Greek, and mean “different” and “affinity”, respectively. The Taber’s Medical Dictionary defines them as an antibody response to an “antigen other than the specific one” (3). In 1973, a classic paper identifying endogenous antibody interference was published by Prince et al. (4), who demonstrated the occurrence of false-positive results in the two-site immunometric assay for hepatitis B antigen. The interference was produced by the bridging of guinea pig antibodies used as capture and detection antibodies in the assay by a human antibody. Of note is that these investigators showed that the interference could be simulated to varying degrees by antibodies from several species and could be reduced by treating the sample with nonimmune guinea pig globulin.

In 1980, Hunter and Budd (5) showed that a two-site immunoradiometric assay for α-fetoprotein showed substantial interference as a result of bridging by human anti-sheep antibodies. The specimen showed much less interference in a competitive binding assay, suggesting that the interference was produced by weak antibodies that did not compete well with a specific antigen, but could bridge the capture and detection antibodies together in a two-site assay.

This type of interference was increasingly designated as heterophile antibody interference (6–9). After the development of monoclonal antibody hybrid techniques in the 1980s, a large number of two-site immunometric assays in kit format became available, giving rise to a number of papers describing bridging interferences.

In most cases, heterophile antibodies arise from the natural process of antibody diversity that produces weak, early, multispecific antibodies against diverse antigens (10–12). This process allows for combinations of gene products, giving rise to more than $5 \times 10^7$ different antibodies with different antigen combining sites (13). The genome can direct the synthesis of rudimentary forms of antibodies that combine with nearly all antigens. As antigenically driven cells divide, somatic mutations occur at a surprisingly high rate, leading to highly specific antibodies (13). Antibodies generated early in this process may show a large degree of multispecificity with broad reactivity for many different types of molecules (14, 15).

Several mechanisms have been proposed to explain the structural basis for this phenomenon (15, 16). These multispecific, or polyreactive, antibodies often exhibit properties of autoantibodies, are frequently IgM rheumatoid factor, are more common among neonates, and may be idiotypic antibodies (11, 13–15), which is in accord with the idiotypic-antidiotypic antibody regulatory network theory of Jerne (17). In fact, in his Nobel Prize Lecture, Jerne noted, “we could merely say that the variable region of an antibody molecule displays several equivalent binding sites . . . , and that every antibody molecule is multispecific” (17). An important point that should be recognized is that, for the most part, multispecific antibodies are weak antibodies. A recent review proposed a scenario...
that showed how specific monoreactive antibodies may have evolved from these primordial natural antibodies (16).

Specific HAAAs also interfere with two-site immunoassays by bridging the capture and detection antibodies. We believe it is important to differentiate between interference by multispecific, poorly defined antibodies and by antibodies that are clearly developed against well-defined specific antigens to which individuals are exposed during therapeutic manipulations. The distinction is important because the nature of the antibody and the avidity will help determine the steps needed to identify and reduce or circumvent the interference.

Because of the weak avidity of heterophile antibodies, they can bridge two assay antibodies together reasonably well, but cannot compete well with the high affinity antigens in competitive binding assays. In addition, they generally do not interfere with nephelometric/turbidimetric assays, in which the concentration of analyte being measured is relatively high. Thus, these types of assays may be useful in circumventing the problem.

Because heterophile antibodies are usually multispecific and often show broad activity against nonimmune globulin from several species, one would think they would be easily removed with nonimmune globulin. They may, however, show activity against idiotopes, which are not present in nonimmune globulin (8), or against conjugated detector antibodies (8, 18). As a result, they often are more difficult to remove or neutralize by immunoabsorption techniques than specific HAAAs (6–8, 11, 18). It is of course possible that a specific HAAA can also be directed against a particularly immunogenic specific idiotope found only in the peculiar antibody used for therapeutic purposes and not in related immunoglobulins.

In our opinion, an example of correctly defined heterophile interference can be seen in another recently published case in this Journal, describing endogenous antibody interference with a new troponin I assay (19). In this case, the authors correctly concluded that the interfering antibodies were heterophile because the patient had no obvious exposure to animal proteins in a social or therapeutic setting and the interfering antibodies appeared to be multispecific in that they bridged assay antibodies from two different species—a mouse capture antibody and a goat detection antibody.

To avoid continued confusion in this area, we suggest the following outline to classify endogenous antibody interference:

1) Antibodies should be called heterophile when there is no history of medicinal treatment with animal immunoglobulins or other well-defined immunogens and the interfering antibodies can be shown to be multispecific (react with immunoglobulins from two or more species) or exhibit natural rheumatoid factor activity. Although it is desirable to test the interfering antibody for multispecific behavior, in practice both of the antibodies used in the test are often monoclonal mouse antibodies. In such a case, when there is no well-defined immunogen, we suggest that the interfering antibody be called heterophile.

2) Antibodies should be called specific HAAAs when there is a history of treatment with animal immunoglobulin and immunoglobulin from the same species is used in the test. The endogenous antibody should be classified as a specific HAAA [for example, human anti-rabbit antibody or human anti-mouse antibody (HAMA)]. Clearly, there are situations in which heterophile antibodies will coexist with specific HAAAs. In such cases, their presence could be demonstrated by successive absorptions.

We add the following note of clarification: Bridging antibodies may develop in animal handlers or other persons through the process of coincidental immunization (6). In such cases, it may be unclear whether these are heterophile antibodies or not. The interfering antibodies may have developed toward one immunogen, but the interference may be with a test antibody from a different species because of interspecies cross-reaction among similar epitopes on immunoglobulins. Like heterophile antibodies, these often exhibit the properties of weak antibodies. The major factor in our classification is that of a poorly defined immunogen. If the patient has a history of social animal handling but the antibodies used in the assay are of a different species, then the interference should be classified as heterophile. This is especially important in the cases of many persons that handle domestic animals such as cats and dogs socially, but show activity against mouse, rabbit, or goat immunoglobulins. Specific HAAAs may develop in the case of occupational exposure. If the interfering antibody shows activity against test antibodies from the same species, the interference may be classified as a specific HAAA if the interfering antibody shows a strong avidity for the suspected immunizing species compared with other species. This situation would require additional testing.

We believe the simple scheme outlined above is a reasonable approach that will produce correct identification in the great majority of cases and reduce confusion. The term HAMA is widely used to describe a heterophile antibody that binds mouse immunoglobulins. In fact, heterophile antibodies have been referred to as preexisting HAMAs (20, 21). By adding the term specific to antibodies with clearly defined immunogens, the term HAMA or other HAAA could continue to be used to refer to a heterophile antibody recognized in mouse assays or other animal-specific assays. Furthermore, terminology for describing immunometric assays that are called HAMA assays, which continue to be useful for identifying endogenous antibodies, would still be used appropriately for either heterophile or specific HAMA interference. We encourage further discussion of this matter through correspondence.
Glossary

Antiidiotypic antibody, an antibody that binds to an idiotope. In a practical sense, it is not be possible to distinguish an idiotope from a paratope because the binding sites are equivalent. Thus, in Fab-Fab bridging, it is unclear which antibody is binding and which is being bound.

Epitope, discrete site to which an antibody binds.

Fab, the variable region of an immunoglobulin, containing the antigen combining site and idiotope.

Fab-Fab, bridging of Fab regions of immunoglobulins as a result of idiotypic antibody binding.

Fc, the constant region of an immunoglobulin that gives class specificity.

Fc-Fab, bridging of two immunoglobulins as a result of rheumatoid factor activity.

Heterophile antibodies, a group of antibodies exhibiting multispecificity that react with heterogeneous antigens.

Idiotope, antigenic determinant of Fab.

Multispecific antibody, an antibody that combines, via its antigen combining site, with structurally different antigens.

Paratope, site on an antibody that combines with the antigen.

Polyspecific antibody, same as a multispecific antibody.

Rheumatoid factor, autoantibodies that bind to multiple antigenic determinants on the Fc portion of IgG.

References


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