

Genetics of Childhood Disorders: XXXIV. Autoimmune Disorders, Part 7: D8/17 Reactivity as an Immunological Marker of Susceptibility to Neuropsychiatric Disorders

TANYA MURPHY, M.D., AND WAYNE GOODMAN, M.D.

Sydenham chorea (SC), a major manifestation of rheumatic fever (RF), is thought to occur when antibodies directed against group A streptococcus (GAS) cross-react with epitopes on neurons of the basal ganglia. In earlier work with SC, Swedo and colleagues identified children who, in addition to chorea, presented with obsessive-compulsive behavior. A precipitous onset of childhood obsessive-compulsive disorder (OCD) after streptococcal pharyngitis was subsequently described that shared many similarities to SC but did not have chorea or clinical signs of RF such as arthritis and carditis. Swedo and colleagues termed this subtype of childhood-onset OCD, *pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections* (PANDAS).

Both pathogen- and host-related factors appear to influence the risk of acquiring RF, with only 2% to 3% of untreated individuals infected by GAS developing RF. Susceptibility to RF is influenced by age, GAS serotypes, family history, and environmental conditions. Children between the ages of 5 and 14 years show the highest rate of this complication. The observation that RF is more prevalent among relatives of the probands than unrelated controls supports the hypothesis that susceptibility to RF is, in part, genetically determined. Environmental

influences, such as crowded living conditions, may contribute to the risk of developing RF. Pathogen-mediated factors play a role as well, with specific GAS serotypes conferring increased susceptibility to RF, although genome-based analyses of GAS should lead to identification of more specific virulence factors. In the absence of carditis and arthritis, the diagnosis of SC is frequently a diagnosis of exclusion. Elevated streptococcal titers at the time of presentation suggest but do not prove a causative role. Similarly, the association of streptococcal illness with children presenting with PANDAS may occur coincidentally. Advances in identification of reliable clinical and/or biological markers of these disorders could further our understanding of pathophysiology and lead to increased specificity in diagnosis and treatment.

Progress toward identifying a molecular marker for RF began in the 1970s, when Patarroyo and colleagues isolated an antibody from the serum of a multiparous woman with several children who had developed RF. This alloantiserum 883 reacted with B cells from 71% of patients with RF and 16% of controls. Later, Zabriskie and colleagues produced two monoclonal antibodies by immunizing mice with B cells from known 883-positive and 883-negative RF patients, which, when used in

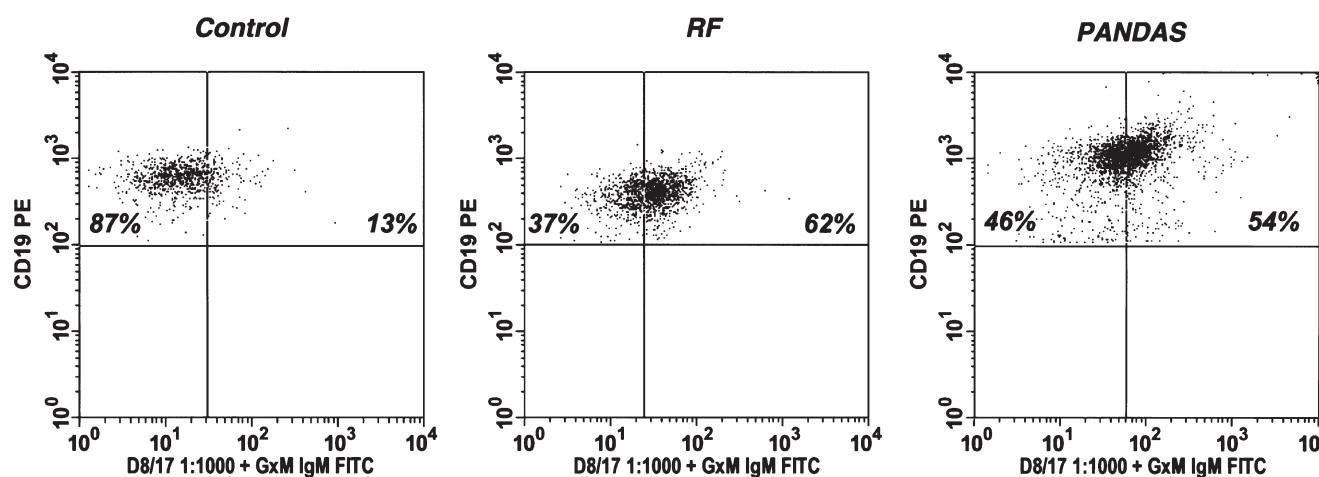


Fig. 1 Dot plots from flow cytometric analysis. Each dot represents a cell. For clarity, only the B cells from each blood sample are shown, based on their labeling with CD19 antibody (vertical axis; B cells fall only in the top half of each plot). Reaction with D8/17 antibody is shown on the horizontal axis. The line separating each graph into left and right portions is drawn based on an internal control sample. Cells to the right of the line are scored as being "D8/17" positive. RF = rheumatic fever; PANDAS = pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections.

combination, identified most patients with RF. Later the monoclonal antibody D8/17 (mAb D8/17) was developed; it reacts with epitopes expressed on expanded populations of B lymphocytes from the majority of patients studied with documented RF, whether 883-negative or 883-positive. This finding was remarkably consistent across the five different geographic and ethnic populations tested.

However, recent studies suggest that the discriminatory ability of D8/17 expression may be reduced secondary to antigenic variation related to ethnicity. Research on groups of patients with RF in northern India has found a lower percentage of D8/17 positivity (62%–68%) and has found additional B-cell epitopes that demonstrate more specificity. These differences may be secondary to methodological, genetic, or environmental variations at the different locations. Although some studies suggest that certain class II MHC haplotypes are increased in patients with RF, D8/17 positivity was not shown in one small study to associate with HLA classification. Larger studies with improved technology would provide a more definitive conclusion.

From these early studies, mAb D8/17 was thought to recognize a constitutively expressed antigen marker on antibody-positive B cells, and it was thought that the level of D8/17 expression remains stable for years after subsidence of active RF. However, levels of expression can be modified under certain conditions; for example, corticosteroid treatment of RF patients has been found to produce down-regulation. Moreover, transient increases above an already elevated baseline occur during acute episodes of RF. No relationship between D8/17 binding and disease characteristics, severity, or acuity has been reported. One exception is a report of decreased percentage of D8/17 positivity in patients with rheumatic chorea (~80%), compared with those with other RF signs (~100%), leading the authors to conclude an element of nosological uncertainty exists with SC.

How the antigen D8/17 relates to the disease process is not understood. Research suggests that it may serve as a trait marker for RF susceptibility. For example, earlier studies with D8/17 suggest that increased binding is specific to type of the poststreptococcal sequelae, as patients with poststreptococcal glomerulonephritis did not demonstrate increased numbers of D8/17-positive B cells. Several other immune-mediated diseases have not shown increased binding. Further evidence to support that the cellular ligand to which D8/17 binds may be constitutive is that increased binding is seen in 96% of RF/rheumatic heart disease patients, 40.3% of unaffected siblings and parents of RF relatives, and 6.7% of healthy controls. Other studies report similar increases in relatives, especially female relatives. Antibodies to these “rheumatic” antigens would suggest that these relatives are at risk for subsequent development of RF; however, RF has been reported in only one relative with a previously demonstrated increase in binding to B cells. Although D8/17 expression appears increased in relatives, its predictive value for risk of RF has not been determined.

The molecular characteristics of the epitope to which mAb D8/17 binds and its role in the pathophysiological process of RF are largely unknown. Based on cross-reactivity experiments using the D8/17 antibody, studies attempting to identify this B-cell antigen suggest homology to helical coiled-coil molecules such as myosin, tropomyosin, and M6 protein of group A hemolytic streptococcus. From this earlier work, many human organs and tissues appear to express antigen reacting with D8/17. Strong binding has been reported to smooth muscle, whereas weak fluorescence in the cytoplasm of cortical and caudate neuronal cells has been detected. One interesting study suggested that compartmentalization of B cells occurs with patients with RF demonstrating the D8/17 antigen on peripheral B cells but not tonsillar B cells, whereas the D8/17 antigen was present on the tonsillar B cells from children without RF but not seen in peripheral B cells. No other work has been reported on D8/17 expression within various areas of the central nervous system or on the characterization of the putative antigen.

Khanna and colleagues conducted a study examining markers that indicate immune activation and found no correlation with D8/17 binding. This finding led them to conclude that the expansion in the number of B cells expressing this antigen does not seem to reflect nonspecific B-cell activation in response to infection. Many more cellular activation markers have been identified in recent years, and comparison with D8/17 binding has yet to be done. In our preliminary work, the intensity of D8/17 binding correlated with duration of illness and was largely attributed to those patients with concurrent comorbid OCD, tics, and attention-deficit/hyperactivity disorder. Increased binding in those patients with a more chronic illness may reflect longstanding effects of altered immune status or genetic up-regulation of a cell surface protein, among many possibilities. A similar finding was reported in which increases of D8/17 expression were observed with increasing age up to the fifth decade. Of interest, a Russian study examining mAb D8/17 binding to B cells in newborn relatives of patients with RF found no binding; this finding suggests that up-regulation occurs sometime postnatally. Another study related increased D8/17 binding to Na^+/H^+ antiporter activity. This antiporter activity was increased in patients with RF and rheumatic heart disease compared with healthy controls and patients with atherosclerotic heart disease. The significance of how this relates to pathogenesis is not known, but Na^+/H^+ antiporter is essential for the maintenance and regulation of cell volume and intracellular pH.

As the diagnosis of SC is often a diagnosis of exclusion, increased expression of D8/17 has been proposed to help differentiate SC from other forms of chorea. Subsequently, the possibility of an immune-mediated pathogenesis of OCD/Tourette syndrome (TS) has generated interest in the potential of monoclonal antibody D8/17 to identify patients having, or at risk for, streptococcus-precipitated neuropsychiatric disorders. Of the studies published to date, increased rates of binding of this mon-

oclonal antibody to B cells has been reported in patients meeting criteria for PANDAS, childhood-onset OCD/TS, OCD (but not trichotillomania), anorexia, and autism. The diagnostic specificity of this antibody and its relationship to the pathophysiology of psychiatric disorders have yet to be established. We are also aware of unpublished studies wherein group differences were not obtained. Although D8/17 binding is specifically increased in patients with RF when compared with other rheumatic illnesses, assessment among several neuropsychiatric conditions is needed to confirm diagnostic specificity of D8/17.

Most of the D8/17 studies have used fluorescein isothiocyanate-conjugated goat anti-mouse antibody to label mAb D8/17 and phycoerythrin-conjugated murine anti-HLA-DR antibody to label B cells. In other words, all the B cells label red and D8/17-positive B cells double-label green and red. The cells are counted using a double-filter microscope, and the proportion of green cells to red cells yields the level of D8/17 expression (typical range 5%–40%). Most studies have defined 11% or 12% mAb D8/17 binding as the threshold for positivity. This threshold ideally is based on the upper 95% confidence limit of the healthy control population and is expected to vary depending on such factors as the intrinsic differences of the control population and assay technique unless standardized. Further studies are also needed to determine sensitivity and specificity of the assay as well as developing appropriate standardization of reagents and technique. At this time, variability in the methodology limits the generalization of conclusions.

MAb D8/17 is an immunoglobulin M (IgM). Antibodies in this class have more avidity or stickiness, and because of this, IgM antibodies are routinely more difficult to use in assays than IgG monoclonal antibodies. This characteristic could contribute to the possibility that the monoclonal antibody is binding nonspecifically to a variety of cell surface proteins that may be up-regulated for unrelated reasons. Identification of the antibody's ligand should provide further insights and improvements in technique. Recent modifications in methodology have led most researchers to use flow cytometry and a more specific antibody for B cells (CD19). Chapman and colleagues have published a flow cytometric analysis comparing samples from patients with OCD/TS to samples from controls and found increased binding of this antibody to B cells in patients. Our experience with flow cytometry suggests that distinct populations of D8/17-positive B cells are not present but binding of mAb D8/17 to B cells is on a continuum. Figure 1 shows examples of flow cytometry dot plots from three subjects. The percentage in the upper-right quadrant represents level of D8/17 positivity. Additional improvements are needed such as appropriate positive and negative controls to use for instrument calibration, a matched isotype control, and standardization of instrument settings, antibody dilutions, and reagents. Our recent work with flow cytometry methods and MAb D8/17

has found many challenges with standardizing this assay, although group differences persist.

With methodological refinements, further work with D8/17 may lead to more definitive results, thereby advancing our understanding of whether some forms of childhood-onset OCD/tics share susceptibility with RF. When we first embarked upon this work, we assumed that an established body of research in rheumatology could be applied to OCD and TS. Along the way we have discovered that much of the early research needed to be reexamined using contemporary immunological principles and procedures. At this time we would advise caution against firm incorporation of monoclonal D8/17 data to establish diagnosis or treatment trajectories in childhood neuropsychiatric disorders.

WEB SITES OF INTEREST

<http://www.niaid.nih.gov/factsheets/strep.htm>
<http://www.nami.org/youth/ocdstrep.html>

ADDITIONAL READINGS

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Dr. Murphy is Assistant Professor and Director of the Child Anxiety and Tic Disorder Clinic and Dr. Goodman is Professor and Chairman of the Department of Psychiatry, University of Florida, Gainesville.

Correspondence to Dr. Lombroso, Child Study Center, Yale University School of Medicine, 230 South Frontage Road, New Haven, CT 06520; e-mail: Paul.Lombroso@Yale.edu.

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