The human immune response is highly complex, dictated by incompletely understood genetic and environmental factors, and it appears to be important in contributing to several forms of cerebrovascular disease. Antiphospholipid-protein antibodies (aPL) have been implicated in immune-mediated clotting in both arterial and venous beds and are considered to be the most common hematological condition associated with ischemic stroke. However, given the vast number of conditions associated with aPL production, including systemic lupus erythematosus, HIV, syphilis, other infections, malignancy, immunizations, and medications, it is clear that aPL are quite a heterogeneous family of immunoglobulins that vary in specificity, isotype, subclass, titer, and associated mechanisms of action. aPL may also be seen in otherwise healthy individuals. Thus, there has been great difficulty in sorting out the potentially important aPL from the nonspecific immune system “noise.” A major breakthrough in the field came in 1990 when 3 groups identified the need for a cooperative phospholipid binding protein in order to detect most, but not all, aPL antibodies. Based on limited data, it has been suggested that infection-related aPL may be less pathogenic and that these aPL do not require a cofactor (apolipoprotein H, also called β2 glycoprotein-1 [β2GP1]) for their detection with ELISA. β2GP1 is a cationic plasma glycoprotein with a molecular mass of 50 kDa and a plasma concentration of ~200 μg/mL. Cofactor-dependent aPL may allow identification of a more specific group of antibodies involved in thrombo-occlusive disease. These differences in aPL may also contribute to the lack of consistent data on their relationship to ischemic stroke (IS).

Two new, intriguing, and important pieces to the aPL puzzle appear in this issue of Stroke. Both articles attempt to tease out the more specific from the nonspecific aPL using a nested case-control study design. A prospective study using cofactor-dependent anticardiolipin antibodies (aCL) has shown that these antibodies are independently associated with incident stroke and myocardial infarction (MI). The other article suggests that aPL may be expected to follow seasonal patterns hypothetically related to aPL produced in response to different stimuli. These 2 large, well-conducted epidemiological studies, in addition to the basic research supporting pathogenicity of aPL in thrombosis, support the role of aPL as potentially important markers/causes of increased vascular risk.

A Significant, Albeit Modest, Risk
Brey and colleagues have applied a key scientific advance in the field, namely, differentiating cofactor-dependent from cofactor-independent aPL, to the study of a large prospectively followed cohort. Men in the Honolulu Heart Study (HHS) were followed up for ~20 years after their sera had been taken and frozen at baseline entry. Japanese-American men who subsequently went on to have an IS or MI had their aPL assay results compared to a group of men in the study who did not develop either ischemic condition. IgG β2GP1-dependent aCL were significantly associated with IS and MI. Despite several case-control studies that have supported a link between aPL and first IS, this association has not been previously documented in a large prospective study. They also studied β2GP1-independent aCL and antibodies to β2GP1 (anti-cofactor antibodies), neither of which was significantly associated with IS/MI. There was no support for a dose-response relationship between any isotype and risk of an event. Using other titer values for positivity cutoffs did not affect the results. Given the long-term follow-up after samples were collected, it could be determined that IS/MI that occurred earlier in follow-up were more strongly associated with aCL than more remote events. Also, IgG β2GP1-dependent aCL appears somewhat stronger as a risk factor for stroke in older subjects rather than in younger subjects. Clearly, this study needs corroboration in women, who suffer higher autoimmune disease rates than men.

The odds of IS (adjusted relative odds in persons with IgG β2GP1-dependent aCL) were 2.2 (1.5 to 3.4) at 15 years and 1.5 (1.0 to 2.3) at 20 years. For MI, adjusted relative odds were 1.8 (1.2 to 2.6) at 15 years and 1.5 (1.1 to 2.1) at 20 years. Thus, cofactor-dependent antibodies are a relatively easy to obtain marker of future risk; however, the risk that it portends may be modest. It is of interest that antibodies typically considered more nonspecific (cofactor-independent) were >10 times less prevalent than the more specific cofactor-dependent antibodies. The lack of a relationship between these cofactor-independent antibodies and IS or MI could be related to the smaller prevalence and lower power to detect such a relationship.

A clear strength of the study is the prospective nature and long follow-up period, yet changes in more classical risk factor status and aCL status over time (possibly a disproportionate prevalence in either group) may have affected the outcome of an ischemic event and/or diluted the effect of a less-potent risk factor such as aCL. Yet, why the impact of
aCL weakens with longer follow-up can only be speculated, as can the more pronounced risk of stroke with aCL in nonsmokers and more pronounced risk of MI with aCL in smokers. These points along with the lack of a dose response could call into question a strong cause-and-effect relationship.

**A Novel Explanation: Hint of a Seasonal Effect**

The other article that addresses potentially different types of aPL takes a novel approach—testing seasonal variability of aCL and antiphosphatidylserine antibodies (aPS). Again using a nested case-control study, aCL and aPS titers were obtained from ~900 IS patients and >1000 controls over 7 years. Striking seasonal differences in the proportion of positive titers were found in controls, possibly mirroring the seasonality of certain infections. The authors suggest that the origins of aPL in individuals with and without IS may differ.

A major contributor to the increased odds of stroke in patients with aPL may be the lower frequency of aPL in controls during the summer months. Statistical “noise” (variance) due to increased production of nonpathogenic aPL may have reduced the potency of the relationship between aPL and stroke during the non-summer months.

One would expect both IS patients and controls to have similar frequencies of nonpathogenic aPL throughout the year. Thus, IS patients would be expected to have higher frequencies of aPL during the winter (nonpathogenic and pathogenic aPL) compared with the summer (only pathogenic aPL). However, this was not found in the study. Perhaps, the important trigger of pathogenic aPL occurs more commonly during the summer (and not the winter).

The stimuli that trigger aCL and aPS may not be identical (they are not perfectly concordant5), and there was no significant seasonal trend for aPS IgM (possibly due to a weaker power of subgroup analysis). A caveat is that these data were based on the month that the antibody was drawn, so the weaker power of subgroup analysis. The authors point out that antibody production was triggered. There are no direct data on the seasonal infection rates in the community of controls or IS patients during the period of study. It will be important to confirm this study in other cohorts and with collection of data related to infection such as fever and measures of viral and other infections.

It has been suggested, both in humans9 and in primates, that there is a seasonal variation in the actual immune response, and this may perhaps be related to the findings of the second study. The role of infection-related aCL as a potential trigger of IS and clotting has been previously addressed both clinically12 and experimentally13 and infection in the week prior to IS is not uncommon. If this novel finding of a seasonal aPL pattern is confirmed, it would add one more variable (to a growing list) to consider in study designs that target ascertaining the role of aPL in thrombo-occlusive events.

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