

# Immunoglobulin G Subclass Profile of Anticitrullinated Peptide Antibodies Specific for Epstein Barr Virus-derived and Histone-derived Citrullinated Peptides

To the Editor:

Studies have shown that the anticitrullinated peptide antibodies (ACPA) response is highly polyclonal, in terms of epitope specificity, V genes, and isotype usage<sup>1,2</sup>. Longitudinal studies of patients with rheumatoid arthritis (RA) have documented epitope spreading, and ACPA, specific for distinct citrullinated epitopes, have been described. By using different citrullinated antigens, ACPA from immunoglobulin (Ig)G, IgA, and IgM isotype have been detected<sup>3</sup>. ACPA are polyclonal in the usage of different IgG subclasses, but in this case the pattern is more heterogeneous. So far the studies conducted indicate the dominance of IgG1 and IgG4, while IgG3 have been detected with cyclic citrullinated peptide (CCP) and vimentin, but not with fibrinogen<sup>4,5</sup>.

The production of specific IgG subclasses might help in deciphering the mechanisms eliciting B cell expansion in response to different antigens. Thus, it is of interest to explore the profile of IgG subclasses of antibodies reactive with novel citrullinated substrates, already known to be tools for ACPA detection.

Ninety-three patients with RA, 25 with psoriatic arthritis, 15 with ankylosing spondylitis, and 48 healthy controls were recruited and ACPA were detected by using an ELISA with 4 synthetic peptides derived from viral proteins<sup>6</sup> (viral citrullinated peptide; VCP) or from histones<sup>7</sup> (histone citrullinated peptide; HCP). Peptides containing arginine were used as the control. Anti-IgG alkaline phosphatase-coupled antibodies and biotin labeled isotype-specific monoclonal antibodies were used.

Results were expressed as percentage of an internal positive control and levels higher than the 97.5th percentile of normal sera were considered positive. Clinical evaluation was performed at the same time as blood sampling in 51 out of 93 patients.

IgG ACPA were detected in 66%, 67%, 57%, and 63% of the sera by VCP1, VCP2, HCP1, and HCP2, respectively. IgG1, IgG3, and IgG4 ACPA were found in variable percentages of RA sera, while the number of sera containing IgG2 was negligible. In fact, IgG1 ACPA were detected in 35%, 40%, 30%, and 43% of the sera; IgG2 in 8%, 1%, 1%, and 11%; IgG3 in 52%, 58%, 23%, and 61%; and IgG4 in 45%, 55%, 18%, and 40% by VCP1, VCP2, HCP1, and HCP2, respectively. Disease control sera did not contain ACPA of any isotype (data not shown). RA sera and normal controls have also been tested on peptides containing arginine. Antibodies of IgG3 and IgG4 isotype were not detected; only IgG1 antibodies specific for arginine containing VCP2 were found, but they were present in similar amounts in both normal and RA sera (data not shown).

The presence of antibodies in at least 1 subclass was comparable with HCP2, VCP1, and VCP2 (71%, 76%, and 80%, respectively), but lower with HCP1 (49%). Analyzing the relationship between the levels of IgG1, IgG3, and IgG4, we detected a strong correlation between IgG3 and IgG4 antibodies with all of the peptides ( $p < 0.00001$ ). IgG1 and IgG3 levels were also highly correlated (HCP2 and VCP1:  $p < 0.00001$ ; VCP2:  $p < 0.0002$ ; and HCP1:  $p < 0.02$ ). The correlation was less significant or absent when IgG1 and IgG4 were compared (HCP1:  $p < 0.05$ ; HCP2: not significant; VCP1:  $p < 0.02$ ; and VCP2:  $p < 0.04$ ).

When the coexpression of different subclasses was analyzed (Figure 1), most sera were characterized by the contemporary presence of 2 or 3 subclasses when tested on any peptide except HCP1, which often detected a single IgG subclass.

These results indicate that the subclass distribution of ACPA in RA sera is skewed and not representative of the serum amounts of immunoglobulins in the different subclasses. Using citrullinated peptides derived from viral proteins or histones as probes, only low levels of ACPA IgG2 were detected, even if IgG2 was the most abundant serum IgG after IgG1. Very low levels of IgG2 ACPA, as previously reported with CCP, vimentin, and fibrinogen, seem to be a general feature of the ACPA response.

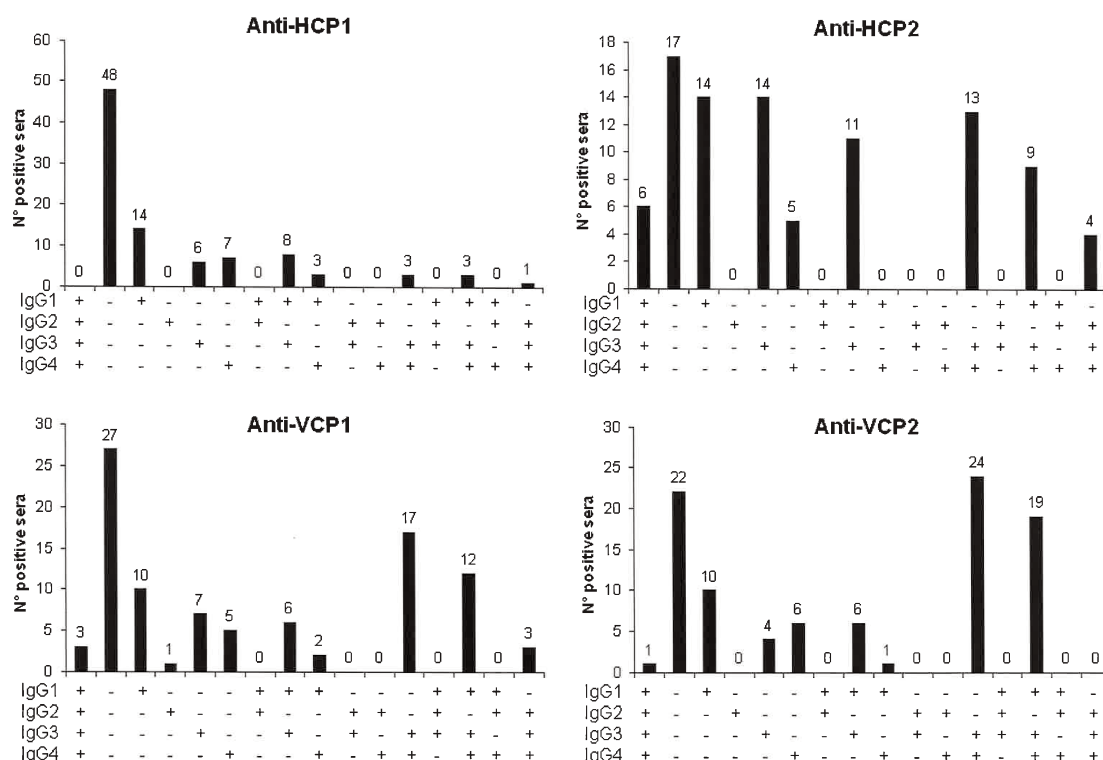


Figure 1. Distribution of antihistone citrullinated peptide 1 (HCP1), HCP2, antiviral citrullinated peptide 1 (VCP1), and VCP2 immunoglobulin G (IgG) subclasses in patients with rheumatoid arthritis.

Similarly, high amounts of IgG4 are found with all the antigens used to measure ACPA. IgG4 are considered noninflammatory antibodies produced after prolonged immune stimulation. However, pathogenic IgG4 have been described in bullous diseases<sup>8</sup> and bispecific antibodies bearing both rheumatoid factor and ACPA activity have been detected in RA<sup>9</sup>. The lack of correlation between IgG4 and IgG1 suggests further that a different mechanism of induction exists in the 2 antibody subclasses, IgG4 being considered Th2-dependent and IgG1 Th1-dependent. As far as IgG3 are concerned, histones and Epstein Barr virus-derived peptides seem to detect ACPA in this subclass in a higher percentage of sera, when compared with CCP, vimentin, and especially fibrinogen. A different inciting antigen might explain this difference, or alternatively, a more “inflammatory” environment in the patients we studied. In fact, it has been reported that the production of anti-H1N1 IgG3 is correlated with levels of proinflammatory cytokines in subjects vaccinated with influenza antigens<sup>10</sup>. However, in the population we studied, the presence of IgG3 ACPA (or the expression or coexpression of ACPA in a given subclass) was not associated with disease severity or with extraarticular manifestations of the disease.

Thus, in undifferentiated arthritis the detection of ACPA subclasses may help in depicting how the immune response to citrullinated antigens is built. Conversely, in established RA, the current data may imply that subclass detection offers no additional tools for disease subtyping.

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