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Comment on "A Role for Immature Myeloid Cells in Immune Senescence"

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Letters to the Editor

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Comment on "A Role for Immature Myeloid Cells in Immune Senescence"

We recently read an article published in *The Journal of Immunology* by Enioutina et al. (1) and found an experimental procedure we believe should be discussed. In this article, as well as in many others by the same authors—also in *The Journal of Immunology* and other renowned journals (2, 3)—serum-specific Abs are quantified by ELISA. According to our knowledge, this could be a misconception, given that we believe ELISA cannot be used for that purpose.

Polyclonal antisera raised against the same Ag could differ in both the affinity and the concentration of the specific Abs. The main premise of quantification is that sample and reference standards must share all biological and physicochemical properties because the analyte concentration is the only unknown factor. The authors have not declared what they have used as the "reference standard," not in the article that gives rise to this letter, nor in the others published before. Considering that the samples are polyclonal antisera, we believe there is no way to construct such a standard curve that allows expressing the results as a concentration of specific Igs. Therefore, we think that the only reliable information that can be obtained from this sort of assay is a "relative potency" value, commonly expressed as titer of specific Abs.

We certainly think that this issue does not affect the quality of the work by Enioutina et al., but we do think that the quantitative aspects deserve further discussion.

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Response to Comment on "A Role for Immature Myeloid Cells in Immune Senescence"

Numerous investigators have employed an ELISA to evaluate differences in the levels of Ag-binding Abs present in the serum of immunized humans or animals (1–3). However, we agree with the concern raised by Drs. Ferrari, Cela, and Maglio that this type of assay might underestimate the actual quantity of specific Abs present in serum samples, through a possible loss of low affinity Abs during the exhaustive washing procedures needed to eliminate nonspecific background. For our evaluation of Ag-specific IgG Abs in serum, a reference curve was created with defined concentrations of purified murine IgG plated onto wells coated with affinity-purified goat anti-mouse IgG (H+L chain–specific) Abs. This was followed by treatment with secondary HRP-labeled goat anti-mouse IgG (γ-chain–specific) Abs. Serial dilutions of test serum samples collected from young and old OVA-immunized animals were then plated onto OVA-coated wells followed by treatment with HRP-labeled goat anti-mouse IgG (γ-chain–specific) Abs. The reference curve was then used to report Ab quantity. This protocol is always validated for specificity using serum samples collected from naive/unimmunized mice (negative control) and with hyperimmune serum collected from mice repeatedly immunized with OVA (positive control). Use of this ELISA protocol to quantify Ag-specific Abs offers the additional advantage, with minor modification, of being able to detect differences in relative potency of various Ab classes and subclasses present in serum or mucosal secretions of immunized animals, thereby providing a means to evaluate influences of distinct adjuvants or therapeutic treatments, or immune modulators on the nature of elicited immune responses. We thank Drs. Ferrari, Cela, and Maglio for their concern; however, reporting "quantity" or "relative potency" does not alter the differences being observed in specific Ab levels between experimental groups.

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