Clin Chem Lab Med 2011;49(5):759–760 © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2011.150

Editorial

Circulating macrocomplexes: old wine in new bottles?

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The existence of macroenzymes, or high-molecular-mass forms of enzymes, has been known for 45 years, since the first report of macroamylase (1). Subsequent reports of macro-lactate dehydrogenase in 1967 (2) and macro-alkaline phosphatase in 1975 (3) established that the conversion of serum enzymes to high-molecular-mass forms is a general phenomenon.

In a seminal review by Remaley and Wilding (4), the authors recognized the existence of immune-enzyme complexes for nine of the most commonly measured enzymes. In particular, class G immunoglobulins (IgG) were found to be involved in the binding of acid phosphatase (EC 3.1.3.2, AP), alanine aminotransferase (EC 2.6.1.2, ALT), alkaline phosphatase (EC 3.1.3.1, ALP), aspartate aminotransferase (EC 2.6.1.1, AST), creatine kinase (EC 2.7.3.2, CK), γ-glutamyltransferase (EC 2.3.2.2, GGT) and lipase (EC 3.1.1.3, LP), while class A immunoglobulins (IgA) were found to bind amylase (EC 3.2.1.1, AMY) and lactate dehydrogenase (EC 1.1.1.27, LD). Class M immunoglobulins (IgM) were found to be rarely involved and no report has been found for IgE- or IgD-mediated macroenzyme. In the initial investigation of macroamylase, it was speculated to exist as an immune-enzyme complex because of its comigration with the γ-globulin fraction during protein electrophoresis, but more sophisticated and elegant experiments using dissociation under acid conditions, precipitation with specific antisera, gel filtration, ultracentrifugation, and papain treatments clarified the nature of these immune-enzyme complexes. Two classic models of autoantibody formation, the ''antigen-driven theory'' and the ''dysregulation of immune tolerance theory'' have been postulated to explain the nature of the immune-enzyme complexes. The alteration of a self-antigen that can become coincidentally cross-reacting with an antibody initially formed against a foreign antigen (e.g., animal amylase) has been used to explain the formation of autoantibodies directed against enzymes from damaged tissues, such as CK and LD in patients with myocardial infarction. In this model, it is not a coincidence that the macro forms to amylase and CK are directed against the most infrequent of the commonly observed isoenzymes in serum, i.e., salivary and BB, respectively. The major evidence for the dysregulation of immunotolerance model is the association of anti-enzyme antibodies with other autoimmune disorders and autoantibodies, including anti-enzyme antibodies (5). From a biochemical point of view, a further even if completely different group of macroenzymes has been recognized, the so-called ''non-immunoglobulin-bound macroenzymes''. This is a heterogeneous group of macroenzymes which should derive from the binding with a substrate (hydroxyethyl starch in the case of amylase), aggregation with lipids (ALP, GGT, 5' nucleotidase and leucin aminopeptidase), or self-polymerization (e.g., mitochondrial CK). The last macroenzyme, also called macro CK type-2 was found to be associated with severe liver diseases and disseminated malignancies and it was proposed as a tumor marker (6).

Despite some efforts to better elucidate the nature of the immune-enzyme complexes and to clarify if they directly cause disease or are simply markers for diseases, most studies were directed to evaluate their interference with catalytic methods for measuring enzymes in serum. In fact, it was underlined the importance of recognizing these entities because of the frequent confusion they cause in the interpretation of serum enzyme results (3).

With the developments of immunoassays and the immunoassay revolution, the interest for immune-enzyme complexes decreased. In fact, the use of antibody-assisted techniques for enzyme detection in terms of protein concentration (''mass'') instead of catalytic activity, was supposed to promise a more accurate and interference-free measurement of many enzymes, first and foremost CK-MB, in clinical practice. The ''troponin-era'', therefore, was supposed to give an answer to all clinical needs, including the provision of a reliable laboratory result, not affected by interferences, even from immune-complexes. However, a paper published in this issue of Clinical Chemistry and Laboratory Medicine highlights the evidence of the ''macrotroponins'' which may result in unexpected and clinically unexplainable increases of the cardiac troponin in serum (7). In particular, Michielsen and colleagues describe the existence of a complex between a high molecular weight protein and a fragment of troponin I that is immunoreactive only with a particular immunoassay. This finding is very interesting for several reasons, namely the need to exclude interferences in the case of abnormal values of cardiac troponin in subjects without cardiac diseases even if the immunoassay utilizes antibody configurations recommended by the IFCC Committee on Standardization of Markers of Cardiac Damage (8). This is not the first report of a macrocomplex involving troponins. It was previously reported a macrocomplex involving cardiac troponin I and IgG which resulted to give abnormally high results with several immunoassays because the prolonged half-life of high molecular mass complex (9). Although these reports are nearly a decade old, the incidence may increase in the future, given that some contemporary and ''next gen-

eration'' troponin assays utilize more than two antibodies. Therefore, binding of the autoantibody has less of a chance of obscuring the epitope for the antibodies used as part of the troponin assay itself (a mechanism that could result in a false-negative result).

In a previous issue of the journal, it was published a paper dealing with a very interesting and different type of immunocomplexes, that are IgM antibodies bound to cancer biomarkers, namely the squamous cell carcinoma antigen (SCCA) (10). Previously published studies by the same group demonstrated that tumour biomarkers may lead to the formation of circulating tumour associated antigens-IgM complexes that should be used as biomarkers in several neoplastic diseases such as prostate, colorectal and liver cancer (11). The overall results obtained led the authors to speculate that IgM-biomarker immune complexes could be involved in cancer immunoediting, likely reflecting host immune protective mechanisms to apply selective pressure on neoplastic cells trying to overcome the tumour. Natural human polyreactive IgM autoantibodies in the immune-complexes should bind the tumour antigens with low affinity but with pentameric avidity (12). The Michielsen et al. case report of macro troponin I may represent another yet unknown mechanism that may add another theory to those we have cited here. Cardiac troponin I in serum should not viewed as a ''foreign antigen'', given that with use of high-sensitive assays, there is a measurable baseline concentration of cTnI in all healthy subjects (13). Moreover, there was no evidence of autoimmune disease in this patient, nor was its finding associated with a neoplastic process.

Therefore, while the existence of circulating immune complexes has been recognized more than four decades ago, new insights regarding macrocomplexes involving cardiac troponins confirm the importance of their recognition in the case of clinically unexplainable increased values of this biomarker, thus avoiding the risk of misinterpretation. In addition, the evidence of the existence of circulating immune-complexes between IgM and tumour associated antigens paved the way to a new field of research aiming to evaluate the diagnostic and prognostic efficiency of a new class of circulating tumour markers and to better elucidate the nature of these complexes.

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