Unreliable Measurement of Basophil Maximum Leukotriene Release with the Bühmann CAST 2000 Enzyme-Linked Immunosorbent Assay Kit

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Received 12 October 2005/Returned for modification 8 December 2005/Accepted 9 January 2006

The Bühmann CAST 2000 enzyme-linked immunosorbent assay is a potentially useful assay for measuring sulfidoleukotrienes released in vitro by allergen-challenged basophils. However, we observed that the positive-control reagent yielded positive signals in cell-free systems. These false-positive results depended on using a mouse anti-FcεRI monoclonal antibody and were prevented by degranulation-inducing reagents other than mouse monoclonal antibodies.

Assays measuring sulfidoleukotrienes (sLT) released by blood basophils following in vitro challenge with specific substances are important diagnostic tools for immunoglobulin E (IgE)-dependent and IgE-independent allergies. As for the latter ones, to date basophil degranulation tests are the only available in vitro diagnostic tools for studying a large proportion of adverse reactions to drugs (anesthetics, nonsteroidal anti-inflammatory drugs, and antibiotics) and food additives (2, 7).

The Bühmann CAST 2000 enzyme-linked immunosorbent assay (ELISA) (Bühmann, Schoenenbuch, Switzerland) quantifies sLT released by blood basophils by a procedure suitable to clinical laboratories and has good predictive power for non-IgE-dependent reactions (1, 6).

We observed that the positive control reagent of CAST 2000, indicated by the manufacturer as an activating monoclonal antibody (MAb) to FcεRI, also provoked sLT signals in cell-free samples or in cellular assays devoid of sLT. This applied to reagents belonging to different batches, which were purchased over a 3-year time period.

The principle part of CAST 2000 is covered by a patented technology (U.S. patent no. 5,487,977) and can be only partly extrapolated from the information made available by the manufacturer. Briefly, dextran gradient-enriched leukocytes are extrapolated from the information made available by the man-
Of note, when stimulated with anti-NIP IgE followed by NIP-BSA, two samples (numbers 2 and 8 in Fig. 1) yielded values below the threshold suggested as the lower acceptable limit for maximum degranulation (200 pg/ml).

The main result of the present note is that the positive control of a common diagnostic kit (Bühlmann CAST 2000 ELISA), used to measure basophil degranulation via sLT release, does not provide reliable results, since it also yields a positive signal in the absence of sLT. This problem likely derives from the design of CAST 2000. The presence of the mouse anti-FcεRI MAb in the positive control supernatant causes competition with the mouse anti-sLT MAb towards the solid-phase antimouse IgG antibodies. A diagram of the detection system is shown in Fig. 2. Our hypothesis is supported by the fact that several mouse IgG MAb, with either identical (9E1) or unrelated (OK-T4 and AC38) specificity, reproduced the false-positive result. In contrast, when a human IgE MAb was used, no false-positive results were detected.

Concerning the alternatives for obtaining maximum stimulation, cross-linking human IgE via a multihapten carrier did not interfere with the CAST 2000 detection system. However, the presence of endogenous IgE bound to FcεRI has to be taken into account as a factor potentially limiting the appropriate evaluation of maximum release.

The above-reported method for inducing maximum degranulation faithfully reproduces an IgE-induced activation. However, this might not be easy to transfer to the routine laboratory. A possible alternative would be the usage of the formyl-methionyl-leucyl-phenylalanine peptide in the same reaction condition of the anti-FcεRI antibody. This peptide is a well-known nonspecific leukocyte activator (4), and it has been used in a commercial test (the BASOTEST), based on previ-
ous findings (5). However, several limitations have been reported for its usage, e.g., the fact that it does not mimic an IgE-induced activation. More importantly, the extent of basophil activation with N-formyl-methionyl-leucyl-phenylalanine does not parallel that observed with anti-IgE reagents, and it is often several times lower (3). Dedicated studies on cohorts of proper numerosity are needed to clarify the potential of this and other nonspecific, degranulation-inducing reagents.

The CAST 2000 could potentially be a useful assay for measuring the functional activity of the primary effector cells of allergy. However, the lack of a proper positive control precludes the detection of samples where degranulation already took place, either in vivo (for biological reasons) or ex vivo for the improper handling of the blood sample. Moreover, the correct measurement of maximum versus baseline degranulation could be exploited in unexplored situations of chronic mediator release by basophils, such as chronic idiopathic and physical urticarias.

REFERENCES


FIG. 2. Diagram of the CAST 2000 ELISA and the mechanism for the false-positive result generation. sLT covalently bound to alkaline phosphatase (sLT-AP) competes with sLT present in the sample (sLT) for binding to a mouse anti-sLT MAb. The immune complex (MAb-sLT) is captured by the antimouse antibody coated on the ELISA plate. The signal is inversely proportional to the amount of sLT. In the positive control sample, a mouse MAb (anti-FcεRI) could compete with the anti-sLT MAb for the binding to the antimouse antibody coated on the ELISA plate, inducing a false-positive signal.