IgE-mediated bleomycin hypersensitivity : evidence from drug-reactive T lymphocytes

We present the case of a 31-year-old woman who had received several intralesional injections with lidocaine (Linisol 100 mg/10 mL, B Braun, Melsungen, Germany) and bleomycin (bleomycin sulphate 15.000 U (=15 mg activity, Sanofi, Diegem, Belgium) because of recalcitrant warts. Within 10-15 minutes after the last injection (1 U/mL), she experienced non-life-threatening anaphylaxis with generalized pruritus, conjunctivitis, pre- and parasternal erythema, angioedema of face and eyelids, hoarseness and dyspnea. There was no hypotension, nor bronchospasm. The patient experienced pruritus and erythema at the site of injection. Most of her symptoms resolved 20 minutes after administration of cetirizine 10 mg orally, methylprednisolone 40 mg intravenously and ranitidine 40 mg intravenously. Sadly, no acute serum tryptase was quantified. Prior intralesional injections did not give rise to an unexpected local reaction. Laboratory analyses, carried out 2 years after the acute event on fresh collected blood samples, showed a normal peripheral blood count, baseline serum tryptase of 3.0 µg/L (Immuno-CAP, FEIA method, Phadia Thermo Fisher Scientific, Uppsala, Sweden). Total IgE was 39 kU/L, specific IgE for Hevea latex and chlorhexidine were both negative (<0.10 kUA/L, Immuno-CAP, FEIA). Skin tests included prick tests with latex and prick and intradermal tests with chlorhexidine digluconate and were negative. Skin prick tests (neat solution) diluted intradermal tests (10^{-2} and 10^{-1}) and a graded subcutaneous challenge test with lidocaine (Linisol 100 mg/10 mL; cumulative dose 1 mL) were negative. We deemed it inappropriate to perform skin tests with bleomycin, as intradermal injection of this compound has been shown to be cytotoxic to keratinocytes and eccrine epithelium (1). A CD63-based basophil activation test (BAT) with bleomycin was negative, possibly because of the time interval elapsed between the index reaction and diagnostic work-up. As shown in figure 1, a CD154-based T lymphocyte activation test (hence called CD154-LAT, detailed in the repository and elsewhere (2)), showed a clear T cell response to bleomycin with up to 14% of the cells responding to an optimal stimulation concentration of 7 µM. Expression of CD154 (CD40L) in response to bleomycin on T lymphocytes from 5 healthy control individuals responsive to positive control stimulation remained merely unchanged (<0.08%). In addition, cytokine release of interferon gamma (IFN-γ) and IL-4 were measured. In response to bleomycin (concentration of 7 µM), an increase in IFN-γ was observed (7.26%) in contrast with the control group (<0.01%). We observed aspecific release of IL-4 in both the patient and healthy controls.

Bleomycin is a complex of related glycopeptide antibiotics from *Streptomyces verticullus*. It inhibits DNA metabolism and is used as an antineoplastic, especially for a variety of solid tumors, but also to treat recalcitrant warts. Despite its widespread and frequent use, to the best of our knowledge, documented immediate hypersensitivity reactions, including life-threatening anaphylaxis, to intralesional bleomycin have not been reported.

Our case report highlights that a T lymphocyte activation test has potential to safely document anaphylaxis to bleomycin and benefit elucidation of the underlying mechanism of this reaction. Actually, in the absence of an assay to measure drug-reactive specific IgE (sIgE) antibodies and because of the potential toxicity associated with skin tests for bleomycin, unlike BAT, the CD154-LAT, was capable to document bleomycin reactive T-lymphocytes in a patient with a clinical phenotype of anaphylaxis. Moreover, synthetic interpretation of the clinical presentation (not being drug naive, low dose, extreme close temporal relationship between exposure and onset of the reaction and signs/symptoms suggestive for mast cell activation), together with the demonstration of drug-reactive T lymphocytes in our patient are highly indicative for a mast cell/basophil degranulation via an IgE-mediated process. Despite a negative BAT, we argue that a positive CD154-LAT suggest a specific immune response. As upregulation of CD154 has been shown to reflect specific T cell receptor activation (3). The exact reason for this basophil non-responsiveness to bleomycin remains obscure but could relate to the general low sensitivity of BAT in drug hypersensitivity that further decreases as the time between the reaction and test increases (4, 5) in contrast to CD154-LAT. Also, our case also stresses that appropriate use of the LAT necessitates knowledge about the dynamics of T activation metrics and guidance to guarantee correct execution and interpretation of the results. Clearly, correct execution of the LAT necessitates construction of dose response curves spanning several log-scales to avoid false-negative results, especially for drugs that can induce false-negative results because of cytotoxicity. This new diagnostic aid in immediate hypersensitivity reactions has yet to be validated in lager cohorts, since most studies focus on delayed reactions. Recently, CD154-LAT was shown to be a reliable marker in the diagnosis of amoxicillin allergy. The addition of cytokines IL-4 and IFN-γ to CD154 as a readout increased sensitivity from 47 to 80% without affecting its absolute specificity in 15 patients with immediate amoxicillin hypersensitivity (6).

In conclusion, we report the first case of non-life-threatening anaphylaxis most likely to intralesional bleomycin in whom the demonstration of drug-reactive T lymphocytes suggested the diagnosis. The presence of drug-reactive T lymphocytes pleads for a specific immune response (an IgE-mediated mechanism). This finding shows that utility of a flow-based T lymphocyte activation test extends beyond a diagnostic tool for non-immediate drug hypersensitivity reactions and that further investigation for the role of T lymphocyte activation tests in immediate drug hypersensitivity reactions is warranted.

References

1. Templeton SF, Solomon AR, Swerlick RA. Intradermal bleomycin injections into normal human skin. A histopathologic and immunopathologic study. Arch Dermatol. 1994;130(5):577-83.

2. Van Gasse AL, Ebo DG, Mertens CM, Bridts CH, Elst J, De Puysseleyr L, et al. CD154 (CD40L): A novel aid to document nonimmediate hypersensitivity to amoxicillin or amoxicillin clavulanic acid. Clin Exp Allergy. 2020;50(5):640-2.
3. Bacher P, Scheffold A. Flow-cytometric analysis of rare antigen-specific T cells. Cytometry Part A. 2013;83A(8):692-701.
4. Heremans K, Toscano A, Elst J, Van Gasse AL, Mertens C, Beyens M, et al. Basophil Activation Test Shows Poor Sensitivity in Immediate Amoxicillin Allergy. J Allergy Clin Immunol Pract. 2023;11(2):500-5.
5. Fernández TD, Torres MJ, Blanca-López N, Rodríguez-Bada JL, Gomez E, Canto G, et al. Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. Allergy. 2009;64(2):242-8.
6. Ebo DG, Elst J, Mertens C, Marie-Line M, Athina L, Michel, et al. Dual intracellular staining of CD154 (CD40L) and cytokines at single-cell level: A novel aid to document immediate hypersensitivity to amoxicillin. Clinical & Experimental Allergy. 2023.