

Correspondence

Reply

To the Editor:

We appreciate Baldo's¹ interest regarding our article,² and his observations about both the precise cefaclor antigenic determinant structures and the uncertainty regarding structures attached in the solid phase. Concerning "solid-phase poly-L-Lysine-cefaclor," no mild conditions were used to preserve the integrity of the cefaclor molecule in its conjugate fragmentation, resulting in an undefined mix of structures on the solid phase. Specifically, poly-L-Lysine-solid phases were treated with a cefaclor solution (10 mg/mL) in aqueous buffer at pH 10.2 (0.05 M Na₂CO₃/NaHCO₃). Because different cefaclor-derived structures can be formed after protein binding, we hypothesize that the recognition of one or others would depend on each patient's immunologic system. Thus, we considered that only solid phases containing mixed structures are able to detect different recognition patterns. In fact, our study demonstrated that recognition occurs with inhibitors 2 and 5, although its grade depends on the patient.²

Certainly, the uncharacterized solid phases described above can detect cephalosporin-reactive IgE.³ We also agree that the undefined nature of these conjugates does not contribute to the identification of allergenic structures. Likewise, the uncharacterized mixture of cephalosporin-butylamine conjugates, evaluated through Radio-Allergo-Sorbent Test (RAST) inhibition, showed recognition by IgE specific to different cephalosporins.³ Although these structures are undefined, we could monitor the cephalosporin structural fragmentation after aminolysis, which led to a mixture of compounds for which the R1 chain seems to remain practically unaffected.⁴ On the basis of these structural results, we published several structure-activity relationship studies including precise structures involving the R1 side chain and a fragment of the β -lactam ring conjugated to an amine molecule, whose recognition by IgE specific to different cephalosporins was demonstrated.^{4,5} However, there was poor recognition for equivalent structures derived from α -amino-cephalosporins (structure 2), which motivated us to study the pyrazinone isolated by Venemalm⁶ in very low yield. Thus, to evaluate its immunologic recognition, we achieved its straightforward synthesis,^{2,7} for which other procedures were unsuccessful.

Previous data reported above support the well-established R1 side-chain contribution to β -lactam allergy,³⁻⁵ although we also consider both the heterogeneity of the IgE response to drugs including cephalosporins and the possibility of more than 1 population of anticephalosporin antibodies of different specificity in the same patient. However, we think that protein conjugation of cephalosporins could only take place spontaneously through aminolysis of the β -lactam ring, and whether the resulting structure could suffer or not different fragmentations would depend on each cephalosporin structure; thus, the entire cephalosporin with opened β -lactam structure, or its fragments, including or not the R2 chain are possible.⁵ However, protein coupling via the carboxyl at position 4 or the R2 side chain should not be considered because this conjugation is not spontaneous: only under forced *in vitro* chemical conditions, and using coupling reagents, this conjugation can be achieved.

We certainly do agree with Baldo that *in vivo* immunologic recognition spectra of IgE will not necessarily match the

specificities identified *in vitro* using chemically modified drug conjugates. However, these structure-activity relationship studies^{2,4,5} are the only potential approach for identifying antigenic determinants, although more research is needed on these structures and the involvement of R1 and/or R2.

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