

Systemic allergy to EDTA in local anesthetic and radiocontrast media

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Clinical Implications

- Patients who experience immediate hypersensitivity reactions to parenterally administered medications such as local anaesthetic, radiocontrast medium, and others may be allergic to the EDTA excipient.
- We recommend that, when intradermal testing confirms sensitization to radiocontrast medium or other medications, testing with EDTA should be considered.

TO THE EDITOR:

We describe a patient with EDTA allergy, identified through investigation of reactions to local anaesthetic (LA) and radiocontrast medium (RCM). A 57-year-old man with a history of seasonal allergic rhinitis received a subcutaneous injection of approximately 2 mL of lignocaine 20 mg/mL, with adrenaline tartrate 12.5 mcg/mL (Lignospan Special [LS], Specialites Septodont, Emu Plains, New South Wales, Australia) (which also contains potassium metabisulfite and disodium EDTA [dsEDTA] 0.25 mg/mL) before excision of a cutaneous lesion. The skin had been prepared with Betadine (Sanofi Aventis Consumer Healthcare Pty Ltd, Virginia Queensland, Australia). There was no exposure to latex. Within minutes, he developed palmo-plantar pruritus, intense facial urticaria, and mild facial swelling, with a feeling of panic but no systemic effects.

Intradermal testing and subcutaneous challenge with lignocaine (without preservatives) produced no reaction (Table 1). There was no reaction to subcutaneous challenge with adrenaline tartrate with sodium metabisulfite (Aspen Pharmacare, St Leonards New South Wales, Australia) or to articaine 4% with adrenaline tartrate and metabisulfite (Septanest; Specialites Septodont). Intradermal testing with povidone-iodine 1:100 produced an equivocal result.

A subsequent excision was attempted by using chlorhexidine antiseptic with LS (a total of 4 mL was administered), but a similar reaction occurred before any incision. Chlorhexidine IgE was negative (<0.35 kU/L; Phadia Fluorescence Enzyme Immunoassay, Immunocap 250, code c8; Thermo Fisher Scientific Inc, Franklin, Mass). Deliberate subcutaneous challenge with 2.0 mL LS (no antiseptic) caused generalized flushing and urticaria. Intradermal testing to calcium disodium EDTA (cdsEDTA; Calcium Disodium Versenate; iNova Pharmaceuticals, Chatswood, New South Wales, Australia) 0.3 mg/mL was strongly positive (Table 1), with negative results in 3 healthy volunteers. Subcutaneous challenge with 0.1 mL cdsEDTA (0.3 mg/mL) resulted in mild facial flushing within 15 minutes. Challenge with 0.5 mL produced injection site

erythema, with prominent well-demarcated facial flushing identical to but milder than his previous reactions. Baseline and acute serum tryptase levels were normal.

The patient had a history of generalized urticaria after administration of RCM (Isovue 370; Bracco Diagnostics, Ferentino, Italy) 4 years earlier. The majority of RCM contains EDTA; intradermal testing was performed on our patient with 6 different RCM, including one that does not contain EDTA (Iomeron 300; Regional Health Care Products Group, Rosebery, New South Wales, Australia). When tested at a 1:10 dilution, results were unclear, however, intradermal testing with undiluted RCM resulted in clearly positive results (strong wheal and flare reaction) in all RCM except Iomeron 300 (Table 1).

Basophil activation testing (BAT) was performed on the patient's peripheral blood. Blood was collected into heparinized-saline tubes and analyzed within 4 hours of sampling. Whole-blood samples from either the patient or a healthy control were supplemented with 2 ng/mL recombinant human IL-3 and incubated for 20 minutes at 37°C with indicated concentrations of cdsEDTA or dsEDTA, saline solution (negative control), or anti-IgE antibody (positive control). At the end of incubation time, further activation and/or degranulation was inhibited by immersion of tubes in ice. The following monoclonal antibodies were used for staining: CD63 FITC (BD 557288; BD Biosciences, San Jose, Calif), CD164 FITC (BD 551297; BD Biosciences), CCR3 PE (BD 558165; BD Biosciences), CD107a PE-Cy5 (BD 555802; BD Biosciences), HLA-DR PE-Cy5 (BD 560652; BD Biosciences), CD203 PE-Cy7 (Beckman A66906; Beckman Coulter Australia Pty Ltd, Lane Cove, New South Wales, Australia), HLA-DR PE-Cy7 (BD 560651; BD Biosciences), and CD123 APC (BD 560087; BD Biosciences) in a 5-color flow cytometric assay. Red cells were removed by standard lysis protocol (BD Biosciences). The expression of activation markers was analyzed in HLA-DR⁺/CD123⁺/CCR3⁺ basophil gate by using BD FACS CantoII flow cytometer and BD FACS Diva analysis software.

Significant upregulation of basophil activation markers and percentages of basophils positive for CD63, CD107a, CD164, and CD203c was observed with the patient's (but not control subject) basophils stimulated with cdsEDTA 0.1 mg/mL (data not shown) and 0.3 mg/mL (Figure 1; Figure E1 in this article's Online Repository at www.jaci-inpractice.org) but not at higher concentrations of cdsEDTA. Similar results were observed with 0.3 mg/mL dsEDTA. IgE signalling via FcεR1 is dependent on protein tyrosine kinase-3 (PI3K). The patient's basophil activation in response to anti-IgE was blocked by the PI3K inhibitor Ly249002.¹ Activation in response to EDTA also was blocked, with an identical pattern across all markers, which suggests that EDTA activation of basophils is IgE mediated (Figure 2).

BAT also was performed with RCM that either contained (Conray 280; Mallinckrodt Australia T/A Tyco Healthcare Pty Ltd, Lane Cove, Australia) or not contained (Iomeron 300) EDTA at 1:10 and 1:100 dilutions, based on published methods.² Whereas EDTA-containing RCM upregulated expression of CD63 and CD107a, markers associated with

TABLE I. Results of intradermal and challenge tests

Drug tested	dsEDTA or cdsEDTA concentration (mg/mL)	Intradermal test			Subcutaneous challenge	
		Dilution (M)	Increase in wheal diameter (mm)	Result		
Lignocaine	0	2%, 1:1	0	Negative	1%, 2 mL	Negative
Adrenaline tartrate (with metabisulfite)	0	ND*			1:1,000, 0.2 mL	Negative
Septanest	0	ND*			1:1 0.1, 0.5, then 2.0 mL	Negative
LS	0.25 (ds)	ND*			2 mL	Positive
EDTA	0.3 (cds)	1:1	10	Positive	0.5 mL	Positive
Conray 280	0.09 (cds)	1:1 (0.74)	3	Positive	ND	
Visipaque 320	0.1 (cds)	1:1 (0.42)	4	Positive	ND	
Ultravist 370	0.1 (cds)	1:1 (0.97)	3	Positive	ND	
Optiray 240	0.2 (cds)	1:1 (0.63)	3	Positive	ND	
Isovue 370	0.39 (cds)	1:1 (0.98)	3.5	Positive	ND	
Iomeron 300	0	1:1 (0.79)	0	Negative	ND	

ND, Not done.

Note. Manufacturer information is as follows: Visipaque 320, GE Healthcare Pty Ltd, Rydalmere, New South Wales, Australia; Ultravist 370, Bayer Australia Ltd, Pymble, New South Wales, Australia; Optiray 240, Mallinckrodt Australia T/A Tyco Healthcare Pty Ltd, Australia; Isovue 370, Regional Health Care Products Group; Iomeron 300, Regional Health Care Products Group.

*Adrenaline-containing solutions cannot be used for intradermal testing.

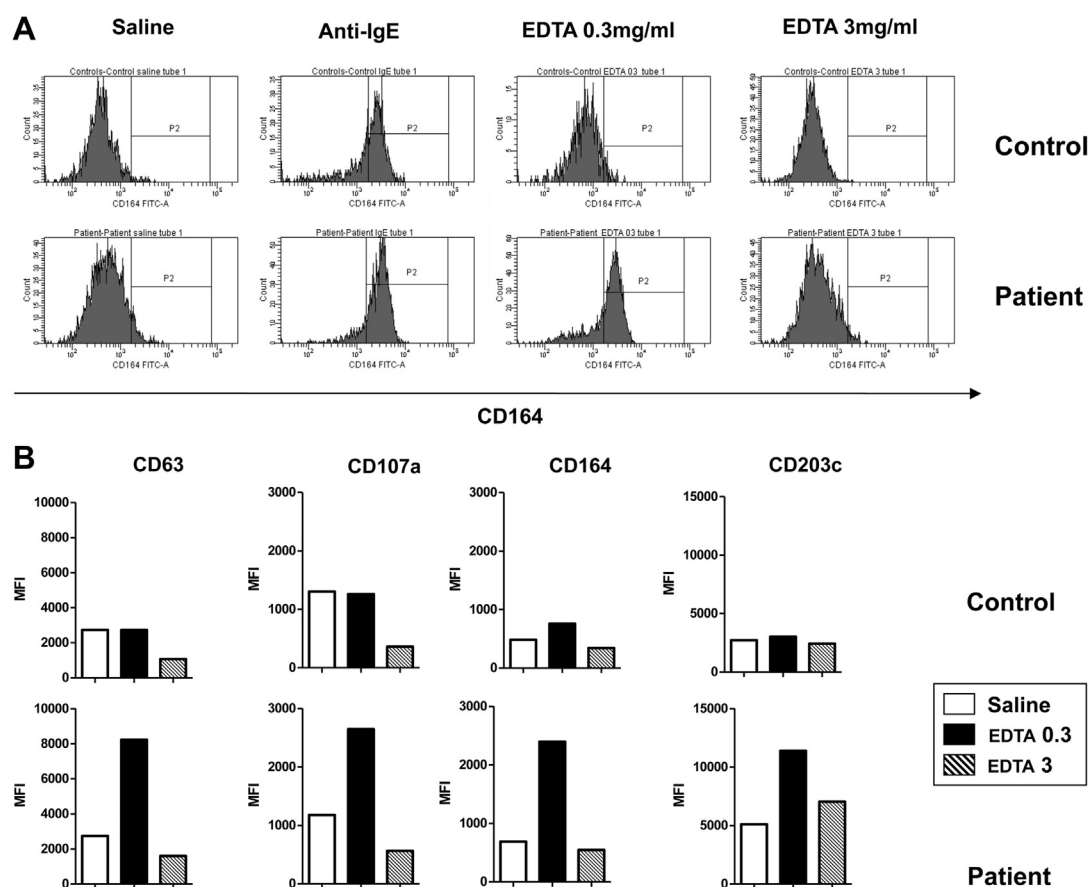


FIGURE 1. Basophil activation markers after stimulation with EDTA. **A**, Expression of CD164 in HLA-DR⁺/CD123⁺/CCR3⁺ basophils from control (upper plots) or patient (lower plots), stimulated with saline solution, anti-IgE antibody, 0.3 mg/mL cdsEDTA or 3 mg/mL cdsEDTA. Representative of 1 of 3 similar experiments. **B**, Mean fluorescence intensity of activation markers on control or patient basophils: saline solution (open bars), 0.3 mg/mL cdsEDTA (filled bars), 3 mg/mL cdsEDTA (dashed bars); 1 of 3 similar experiments.

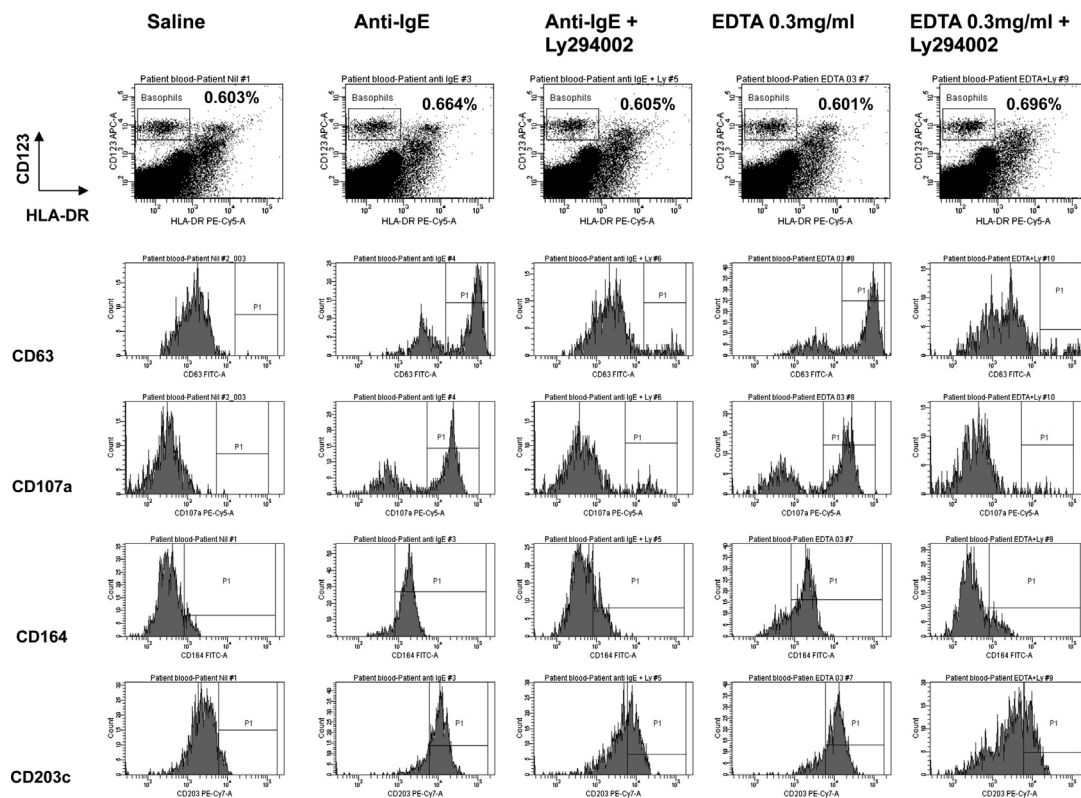


FIGURE 2. Effect of PI3K inhibitor Ly249002 on dsEDTA induced upregulation of basophil activation markers. Whole blood from the patient was incubated for 60 minutes at 37°C with either 20 μ M Ly249002 or vehicle (dimethyl sulfoxide; 1:2500 in saline solution). *Top plots:* percentages of viable basophils for indicated treatment conditions. Histograms, representing CD63, CD107a, CD164, and CD203c expression in basophil gate, are shown in respective order underneath.

histamine release, the EDTA-free RCM did not change the expression of these activation markers (Figure 3), consistent with the intradermal test result, which provided further evidence that EDTA was responsible for the RCM reaction observed in this patient.

EDTA was conjugated to polylysine via carboxylic acid groups by using N-hydroxysuccinimide and 1,3-dicyclohexylcarbodiimide, and was confirmed with UV spectroscopy. EDTA-polylysine and polylysine were used to coat 96-well ELISA plates (Amino-Immobiliser, Nunc, Denmark), which were sequentially incubated with serum, biotinylated anti-IgE (Bioclone, Sydney, Australia) and streptavidin-horseradish peroxidase (BD Pharmingen, Franklin Lakes, NJ), and detected with 1-Step TMB Ultra substrate (Pierce, Rockford, Ill). Negative controls (human albumin and control sera) were included in all experiments. ELISA did not detect EDTA-specific IgE in patient serum.

EDTA is widely used in medicine and industry for its metal-ion chelating activity. EDTA salts have applications in foods, manufacturing, cosmetics, and pharmaceuticals as preservatives and stabilizers, and can be used to treat heavy metal poisoning. The calcium-chelated form of dsEDTA (Na_2EDTA) is cdsEDTA (CaNa_2EDTA). As a nonchelated salt, dsEDTA is able to bind free calcium in serum, whereas cdsEDTA can exchange calcium for iron or lead ions. Notably, dsEDTA is commonly used to block basophil degranulation (at a concentration of 2 mM [0.74 mg/mL]) *in vitro* through the calcium chelating mechanism. A search of a comprehensive drug database revealed 238 oral,

parenteral, and topical medications available in Australia that contain EDTA.³

Contact dermatitis to EDTA has been reported in association with LA, cosmetics, shampoo, sunscreen, hair gel, moisturizers, and contact lens solution, which indicates that it is an immunoreactive molecule,^{4,5} although presumably requiring haptenization. A recent publication described patients with work-related rhinitis who had exacerbations after nasal provocation testing with sodium EDTA, which suggests possible allergy to EDTA, but skin testing was not performed.⁶

To our knowledge, this is the first description of immediate systemic hypersensitivity to EDTA. EDTA allergy is likely to have been responsible for this patient's acute allergic reaction to LA and also to his previous reaction to RCM. Intradermal testing was positive with pure EDTA and with LA, which contained EDTA. Intradermal testing was positive with 5 different RCM that contain EDTA, but negative with Iomeron 300, the only RCM that does not contain EDTA. Intradermal reactivity to RCM correlated with EDTA content and not with osmolality (Table 1), which excluded hyperosmolality as a cause for nonspecific reactions. Whereas a recent review suggested that RCM should be tested at 1:10 dilution,⁷ Torres et al have used undiluted RCM without reporting false-positive results⁸ and our experience is that undiluted RCM produce at most minor swelling without true wheal and flare in nonallergic subjects.

Because EDTA is found in topical as well as parenteral medications, we were concerned about the possibility of reactions

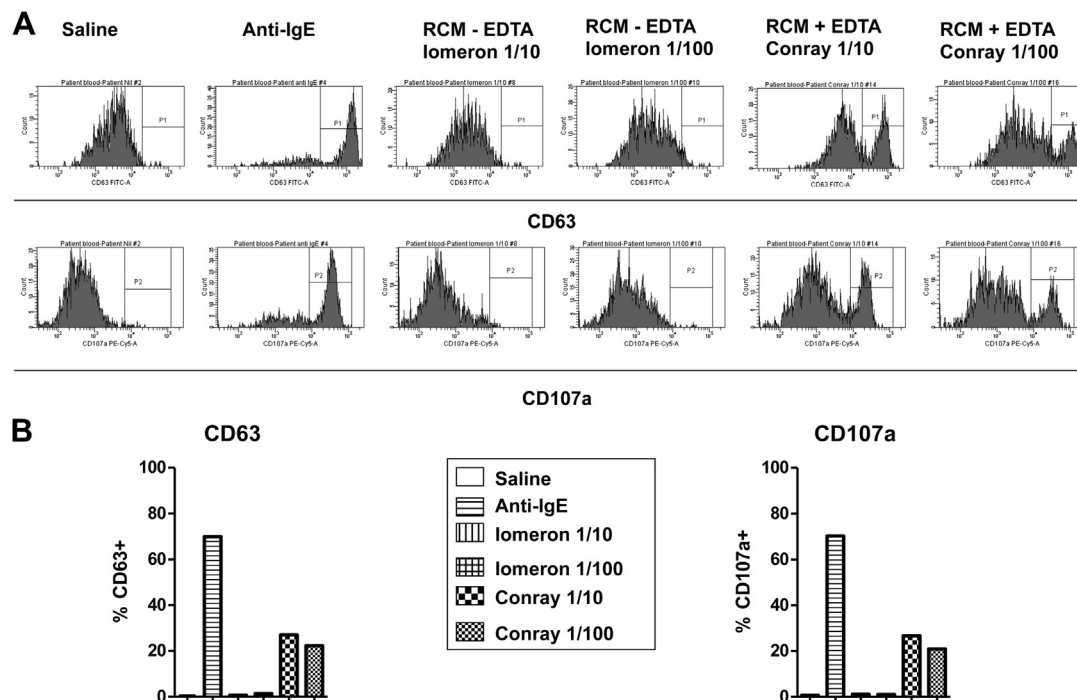


FIGURE 3. Expression of CD63 and CD107a on the patient's basophils after stimulation with RCM with and without EDTA. **A**, Expression of CD63 and CD107a in HLA-DR⁺/CD123⁺/CCR3⁺ patient basophils stimulated with saline solution, anti-IgE antibody, RCM not containing EDTA (lomeron 300) and RCM containing EDTA (Conray 280) at indicated concentrations. **B**, Percentages of patient's basophils positive for CD63 and CD107a after stimulation with saline solution (*open bars*), anti-IgE (*horizontally dashed bars*), RCM without EDTA at 1:10 (*vertically dashed bars*), RCM without EDTA at 1:100 (*hashed bars*), RCM with EDTA at 1:10 (*checkered bars*) and RCM with EDTA at 1:100 (*dotted bars*).

to tablets or eyedrops. However, ocular challenge with eyedrops that contained EDTA was negative with our patient. Of note, human volunteer studies that used radiolabeled cdsEDTA found no radioactivity in the blood after oral ingestion or topical administration, which suggests poor gastrointestinal and trans-mucosal absorption.⁹

EDTA has pharmacologic effects through metal chelation. Bronchial reactivity was demonstrated in an animal model after provocation testing with dsEDTA but not with cdsEDTA.¹⁰ The researchers postulated a pharmacologic mechanism because dsEDTA chelates calcium but cdsEDTA does not. Our patient presented after similar clinical reactions to LA that contained dsEDTA and to RCM that contained cdsEDTA. Intradermal testing was positive with products that contained cdsEDTA, whereas challenge testing produced equivalent results with LA that contained dsEDTA and with pure cdsEDTA. Finally, BAT produced equivalent results with pure preparations of both cdsEDTA and dsEDTA, which indicated that calcium chelation is not the mechanism of action. There is previous evidence for immunologic cross-reactivity of dsEDTA and cdsEDTA in contact dermatitis.¹¹ Overall, this suggests that this patient reacted through immunologic mechanisms (allergy) rather than pharmacologic mechanisms.

Furthermore our analysis of *in vitro* data suggests that the patient-specific basophil reactivity to EDTA described in this case is dependent upon the PI3K-mediated intracellular signaling pathway used by the activated FcεR1.¹ Strikingly, the pattern of activation by EDTA was identical to anti-IgE for all markers, and Ly294002 showed the same pattern of inhibition

for all markers for both anti-IgE and EDTA (Figure 2), which suggests the presence of EDTA-specific IgE, although we did not detect it by our ELISA method. This is feasible because IgE reactivity against related polyamines (EDTA is a polyamino carboxylic acid) has previously been shown.¹² Based on these arguments, an IgE-mediated mechanism can be reasonably proposed. This case has implications for patients with one or more allergies to different classes of parenterally administered medications that contain EDTA. We recommend that, when intradermal testing confirms sensitization to RCM or other EDTA-containing medications, testing for allergy to EDTA should be considered.

Acknowledgments

We thank the patient described in this article, whose own curiosity as to the nature of his allergy complemented ours. The patient consented to all investigations and publication of the case. All *in vitro* testing was performed in accordance with the ethical and confidentiality requirements defined by National Association of Testing Authorities and National Pathology Accreditation Advisory Council, Australia. All healthy volunteers used as controls consented and were deidentified.

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REFERENCES

1. Miura K, MacGlashan, DW Jr. Phosphatidylinositol-3 kinase regulates p21ras activation during IgE-mediated stimulation of human basophils. *Blood* 2000;96:2199-205.
2. Pinnobhoun P, Buranapraditkun S, Kampitak T, Hirankarn N, Klaewsongkram J. The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media. *Ann Allergy Asthma Immunol* 2011;106:387-93.
3. MIMS (Monthly Index of Medical Specialties) Australia, Pty Ltd. Available from: <http://www.mimonline.com.au>. Accessed January 23, 2013.
4. Sánchez-Pedreno P, García-Bravo B, Frías-Iniesta J. Contact allergy to tetra-sodium EDTA in a sunscreen. *Contact Dermatitis* 2009;61:125-6.
5. Pruitt C, Warshaw EM. Allergic contact dermatitis from ethylenediaminetetraacetic acid. *Dermatitis* 2010;21:121-2.
6. Laborde-Castérot H, Villa AF, Rosenberg N, Dupont P, Lee HM, Garnier R. Occupational rhinitis and asthma due to EDTA-containing detergents or disinfectants. *Am J Ind Med* 2012;55:677-82.
7. Brockow K, Romano A, Aberer W, Bircher AJ, Barbaud A, Bonadonna P, et al. Skin testing in patients with hypersensitivity reactions to iodinated contrast media: a European multicenter study. *Allergy* 2009;64:234-41.
8. Torres MJ, Gomez F, Doña I, Rosado A, Mayorga C, Garcia I, et al. Diagnostic evaluation of patients with nonimmediate cutaneous hypersensitivity reactions to iodinated contrast media. *Allergy* 2012;67:929-35.
9. Foreman H, Trujillo TT. The metabolism of C14 labelled ethylenediaminetetraacetic acid in human beings. *J Lab Clin Med* 1954;43:566-71.
10. Lindeman KS, Hirschman CA, Freed AN. Calcium chelators induce bronchoconstriction in the canine lung periphery. *J Appl Physiol* (1985) 1990;68:1114-20.
11. Raymond JZ, Cross PR. EDTA: preservative dermatitis. *Arch Dermatol* 1969;100:436.
12. Zhao Z, Baldo B, O'Brien RM, Plomley RF. Reaction with, and fine structural recognition of polyamines by human IgE antibodies. *Mol Immunol* 2000;37:233-40.

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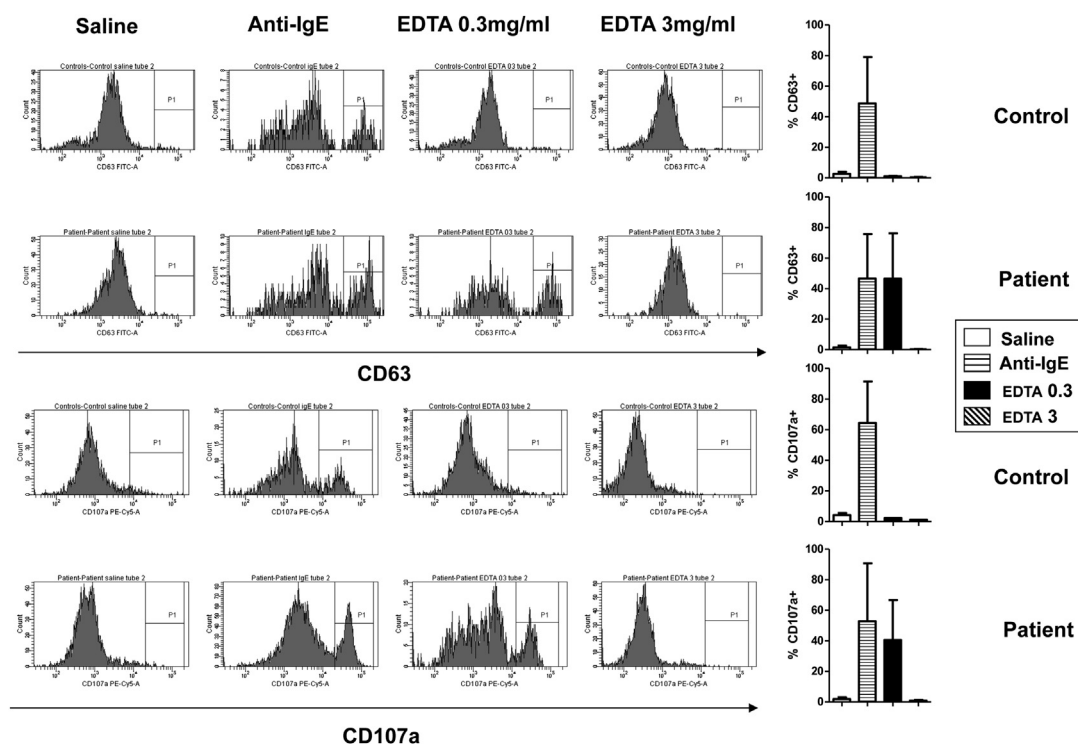


FIGURE E1. Expression of CD63 and CD107a and percentages of CD63 and CD107a positive basophils after stimulation with EDTA. *Histograms:* expression of CD63 and CD107a in HLA-DR⁺/CD123⁺/CCR3⁺ basophils from control or patient stimulated with saline solution, anti-IgE antibody 0.3 mg/mL, or 3 mg/mL EDTA. Representative plots from 1 of 3 similar experiments shown. *Column bars:* Percentages of basophils positive for CD63 or CD107a in control or patient after stimulation with saline solution (open bars), anti-IgE (horizontally dashed bars), 0.3 mg/mL EDTA (filled bars), or 3 mg/mL EDTA (diagonally dashed bars). Data represent mean (SD) from 3 separate experiments.

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