

1 **Title: Rapid desensitization to adalimumab is associated with a decrease of basophil sensitivity**

2
3 **Short running title: Basophil activation test and rapid desensitization to adalimumab**

4
5 **Manuscript word count: 1067**

6 **Figure count: 1**

7
8 **Authors and affiliations:**

9 Jonathan Thévenot, PhD^{1,2}, Marie-Christine Ferrier le Bouëdec, MD³, Anthony Buisson, MD, PhD⁴, Gilles
10 Bommelaer, MD, PhD⁴, Michel D'Incan, MD, PhD^{3,5} and Paul Rouzaille, PharmD, PhD^{1,5,6}

11
12 ¹CHU de Clermont-Ferrand, Service d'Immunologie Biologique, Pôle de Biologie et d'anatomopathologie, 58 rue Montalembert, 69003
13 Clermont-Ferrand, France

14 ² *current address*: INRA / Agrocampus Ouest, UMR 1253, Science et Technologie du Lait et de l'Oeuf, 65 rue de St Brieuc, 35042 Rennes,
15 France

16 ³CHU de Clermont-Ferrand, Service de Dermatologie, CHU Estaing, 1 Place Lucie et Raymond Aubrac, 69003 Clermont-Ferrand, France

17 ⁴CHU de Clermont-Ferrand, Service de Gastroentérologie, CHU Estaing, 1 Place Lucie et Raymond Aubrac, 69003 Clermont-Ferrand, France

18 ⁵ UMR 1240 INSERM/IMoST UCA

19 ⁶ *current address*: CHU de Clermont-Ferrand, Service d'Histocompatibilité, Pôle de Biologie et d'anatomopathologie, 58 rue Montalembert,
20 69003 Clermont-Ferrand, France

21
22 **Corresponding author:** Paul Rouzaille, PharmD, PhD, Service d'Histocompatibilité, Pôle de Biologie et
23 d'anatomopathologie, CHU de Clermont-Ferrand, 58 rue Montalembert, 69003 Clermont-Ferrand, France. Email:
24 porouzaille@chu-clermontferrand.fr. **Phone:** [+334 73 751 983](tel:+33473751983)

25
26 **Conflict of interests:** All the authors declare that they have no relevant conflicts of interest in line with this
27 work.

28
29 **Abstract:** Rapid subcutaneous desensitization to adalimumab has been reported to be an effective management
30 option. Monitoring this protocol using basophil activation test, we report here that desensitization is associated
31 with a decrease of basophil sensitivity.

32
33 **Key words:** adalimumab, rapid desensitization, basophil activation test, CDsens

34

35 Adalimumab is a fully human recombinant monoclonal antibody against TNF- α , largely used in inflammatory
36 diseases, such as Crohn disease or ulcerative colitis. Immediate hypersensitivity reactions to this molecule have
37 been described, either local or systemic, ranging from pruritus to anaphylaxis (1). Rapid subcutaneous
38 desensitization to adalimumab has been reported by several teams, and appears to be an effective management
39 option, especially in patients without other obvious therapeutic options (2–5). Rapid drug desensitization protocols
40 consist in administrating, in the patients who experienced hypersensitivity reactions, full therapeutic doses of
41 eliciting drug, in an incremental manner, step by step (6). Despite no precise consensus exists today concerning
42 the protocol to use, particularly for sub-cutaneous drugs, the sparse cases reported in literature validate the concept.
43 Here, we report that successful desensitization to adalimumab of two patients is associated with a decrease of the
44 basophil sensitivity, that we have monitored using basophil activation test (BAT).

45 The patient #1 is a 30 years old woman, who presents a severe Crohn disease, diagnosed 12 years ago. She
46 experienced severe urticarial lesions at the injection site within one hour of the first injection of adalimumab. This
47 was a new course after a 6-year interruption of treatment (colectomy surgery, pregnancy). The patient #2 is a 38
48 years old woman, suffering from a severe ulcerative colitis, diagnosed 10 years ago. She reported urticarial lesions
49 at the injection site within one hour of the fifth injection of adalimumab. For both patients, prick tests to
50 adalimumab were negative but intradermal test at the concentration of 1:1000 was positive at 20 min, suggesting
51 an IgE sensitization. These two patients were addressed to the dermatology unit to benefit from rapid
52 desensitization protocols to adalimumab. Given the lack of consensus on rapid drug desensitization, protocols have
53 been established in line with the severity of inaugural reactions in these two patients. Patient #1 received for her
54 first cure day a nine-step desensitization protocol, every 30 minutes reaching a cumulative dose of 54,3mg
55 (respectively: 0,005mg, 0,05mg, 0,5mg, 1,25mg, 2,50mg, 5mg, 10mg, 15mg and 20mg). This protocol was
56 repeated every week, decreasing the number of injections to reach only one injection (40 mg) at the visit #10.
57 Patient #2 benefited from a nine-step protocol for the first day (0,005mg, 0,05mg, 0,5mg, 1,25mg, 2,5mg, 5mg,
58 5mg, 10mg and 15mg) with a cumulative dose of 39.30 mg. The protocol was repeated every two weeks,
59 decreasing there again the number of injections to one injection (40 mg), at the visit #12 with a good tolerance.

60 We first report the performance of BAT in the assessment of allergic nature of the reaction (Fig. 1A), using the
61 two most commonly used markers of activation/degranulation: CD203c and CD63. Before the beginning of the
62 desensitization protocol, BAT to adalimumab were indeed strongly positive in both patients, according to the
63 maximal percentage of activated basophil (i.e. CDmax, related to basophil reactivity). We validated the specificity
64 of this *in vitro* reaction by testing four patients treated with adalimumab with a good tolerance. They all display

65 negative BAT to adalimumab (Fig. 1A). Recently, another parameter of basophil activation test, the CDsens, was
66 described to be correlated to basophil sensitivity (7,8). CDsens is defined as $1/LC50 \times 100$, where LC50 is the
67 lowest concentration of allergen giving 50% of the maximum activation of basophils. We thus interested ourselves
68 to investigate CDsens along the rapid desensitization protocol to adalimumab in the two patients. We thus repeated
69 basophil activation testing three times (visits 1, 2 and 3 for the patient #1, *i.e.* before the protocol, at 3 weeks and
70 6 weeks after the beginning; and visits 1, 3 and 5 for the patient #2, *i.e.* before the protocol, at 6 weeks and 10
71 weeks after the beginning) (Fig. 1B). The first finding was, despite the increasing clinical tolerance to adalimumab,
72 BAT remains strongly positive. Indeed, CDmax remain constant, for both patients, using both marker (CD63 or
73 CD203c). However, we observed a diminution of the CDsens parameter, reflecting a diminution of the sensitivity
74 of the basophils, in both patients, with the two basophil activation/degranulation markers. To our knowledge, this
75 is the first demonstration that rapid drug desensitization is associated with modifications on basophils, displaying
76 a higher activation threshold along the injections.

77 Pathophysiological mechanisms associated to rapid drug desensitization are not fully understood today. *In vitro*
78 experiments and *in vivo* mouse models have shown that increasing doses of an antigen lead to prolonged
79 hyporesponsiveness to triggering dose of the desensitizing antigen (6). Recently, BAT has been shown to be a
80 potential biomarker for rapid drug desensitization, and Giavina-Bianchi *et al.* have demonstrated that BAT remains
81 positive upon sequential injection of drug (9). Here we show that, despite the stability of CDmax, rapid drug
82 desensitization is associated to a decrease of CDsens, demonstrating for the first time an impact *in vivo* of such
83 protocols on the sensitivity of human basophils. These preliminary exciting data have now to be confirmed on
84 larger studies, and will help to a better understanding of immunological mechanisms associated to the clinical
85 success of rapid drug desensitization.

86 **References:**

- 87 1. Singh S, Pardi DS. Update on anti-tumor necrosis factor agents in Crohn disease. *Gastroenterol Clin North*
88 *Am.* 2014 Sep;43(3):457–78.
- 89 2. Bavbek S, Ataman Ş, Akıncı A, Castells M. Rapid subcutaneous desensitization for the management of local
90 and systemic hypersensitivity reactions to etanercept and adalimumab in 12 patients. *J Allergy Clin Immunol*
91 *Pract.* 2015 Aug;3(4):629–32.
- 92 3. Gutiérrez Fernández D, Foncubierta Fernández A, Anguita Carazo JL, Fernández Meléndez S, Fernández
93 Anguita MJ, Medina Varo F. Adalimumab desensitization protocol in a patient with a generalized urticarial
94 reaction and angioedema following adalimumab administration. *J Investig Allergol Clin Immunol.*
95 2014;24(4):273–5.
- 96 4. Rodríguez-Jiménez B, Domínguez-Ortega J, González-Herrada C, Kindelan-Recarte C, Loribo-Bueno P,
97 Garrido-Peño N. Successful adalimumab desensitization after generalized urticaria and rhinitis. *J Investig*
98 *Allergol Clin Immunol.* 2009;19(3):246–7.
- 99 5. Quercia O, Emiliani F, Foschi FG, Stefanini GF. Adalimumab desensitization after anaphylactic reaction.
100 *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol.* 2011 Jun;106(6):547–8.
- 101 6. Castells Guitart MC. Rapid drug desensitization for hypersensitivity reactions to chemotherapy and
102 monoclonal antibodies in the 21st century. *J Investig Allergol Clin Immunol.* 2014;24(2):72–9; quiz 2 p following
103 79.
- 104 7. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil
105 activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol.* 2015
106 *Jan*;135(1):179–86.
- 107 8. Chapuis A, Thevenot J, Coutant F, Messaoudi K, Michaud E, Pereira B, et al. Ara h 2 basophil activation
108 test does not predict clinical reactivity to peanut. *J Allergy Clin Immunol Pract.* 2018 Feb 2;
- 109 9. Giavina-Bianchi P, Galvão VR, Picard M, Caiado J, Castells MC. Basophil Activation Test is a Relevant
110 Biomarker of the Outcome of Rapid Desensitization in Platinum Compounds-Allergy. *J Allergy Clin Immunol*
111 *Pract.* 2017 Jun;5(3):728–36.

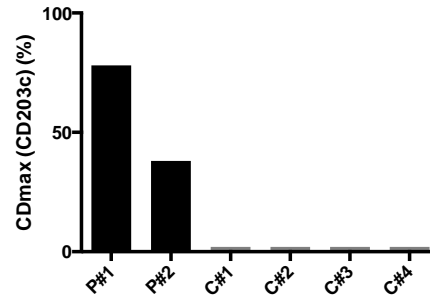
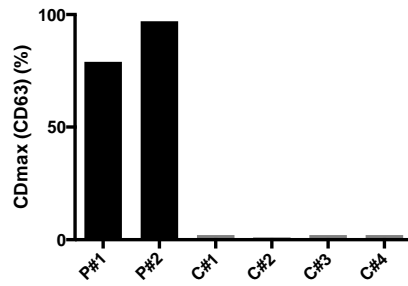
112

113 **Figure legend:**

114 **Fig. 1: Basophil activation test allows monitoring of rapid desensitization to adalimumab.** A. Results of
115 basophil activation test (BAT) to adalimumab, expressed in percentage of maximal activation (CDmax), either
116 using CD63 (left) or CD203c (right), for the two patients (P#1 and P#2) and 4 controls tolerating adalimumab
117 (C#1, C#2, C#3 and C#4). B. Results of BAT for the two patients (up and down), expressed in CD63 (left) and
118 CD203c (right), along the rapid desensitization protocol (at V1, V2 and V3 for the patient #1 and V1, V3 and V5
119 for the patient #2). CDsens is precised for each test.

120

A.



B.

