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Desensitization modulates humoral and cellular immune response to infliximab in a patient with an immediate hypersensitivity reaction

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Clinical implications

Drug desensitization induces a temporary tolerance in patients reactive to monoclonal antibodies. However, its immunological basis has not been completely defined. We show that desensitization for infliximab modifies cellular and humoral response to the drug, by up-regulating drug-specific Treg cells.

Keywords

Drug desensitization; Infliximab; immunogenicity; Treg cells

45 *To the Editor:*

46 This study focused on longitudinally analysing the adaptive immune response to Infliximab (IFX) in
47 one patient during the desensitization procedure to the drug. A 65-year-old man with suppurative
48 hydrosoadenitis, non responder to adalimumab therapy, developed a severe immediate systemic
49 hypersensitivity reaction (HR) to IFX biosimilar (CT-P13), characterized by flushing, throat
50 constriction, low blood pressure (90/60) and low oxygen saturation (sO₂ 90%) (grade 3 Brown
51 classification) (1). The HR occurred at the beginning of the second infusion of a second cycle of
52 treatment, after interruption (1 year) due to a surgical procedure and following suppurative
53 complications. A 16-step desensitization protocol was applied for 580-mg target dose of IFX (Table
54 E1), including premedication with H1 and H2 blockers, systemic corticosteroids and acetaminophen
55 (2). Desensitization cycles were performed according to the induction/maintenance procedure of
56 IFX administration (t0, t15, t45 and every 8 weeks.); the protocol is still ongoing and the patient has
57 received a total of 8 cycles with no adverse reactions. Written informed consent was obtained from
58 the patient for the therapy and the blood sampling.

59 Anti-IFX antibodies (ATI) can increase the risk of hypersensitivity reactions (3), for this reason we
60 tested ATI in our patients. The patient was positive for ATI (non isotype specific anti-IFX antibody,
61 (Promonitor, Progenika Biopharma, Spain) and for anti-IFX IgE, measured by using an home made
62 streptavidin ImmunoCAP assay (Thermo Scientific-Phadia, Sweden) (4). Despite the lack of
63 standardization of skin testing for biologicals, the allergological work up was completed by
64 performing skin testing, accordingly to previously published data (5, 6). Specifically, skin prick test
65 with IFX (10 mg/ml; 1:1) was negative, while intradermal test (IDT, 10 mg/ml) was positive (1:10
66 dilution) (Fig 1A). Skin testing for inhalant allergens was positive for grass pollen, confirmed by
67 detection of serum specific IgE (5.4 kUA/L) (ImmunoCAP).

68 IDT tested positive before the first three cycles of desensitization, but negative after each
69 procedure, confirming previous published data describing negative skin test following

desensitization (7). More importantly, IDT performed at baseline before three following cycles (from 4th to 8th) were negative. Of note, SPT for grass pollen remained positive, before and after each cycle of desensitization (Fig 1A-E). Overall, these findings, firstly provide evidence of a drug-specific mechanism of turning off mast cells operating since the first cycle; secondly, they suggest the decrease/disappearance of an IgE-mediated drug-specific response acquired during repeated courses of desensitization. Looking at the humoral response to the drug, we observed that serum ATI titer (evaluated before each cycle), after a slight increase at the second procedure, progressively declined over subsequent cycles (Fig 1F, left panel). As expected, ATI were undetectable at the end of each cycle due to drug interference in the ELISA assay (Fig 1F, right panel). Accordingly, anti-IFX IgE levels showed similar a trend (Fig 1G, left panel) and in agreement with the *in vivo* tests, anti-grass pollen IgE remain positive during the entire protocol (Fig 1G, right panel). The cellular response to the drug was assessed by evaluating the proliferation to IFX of patient's PBMC collected before each cycle, as described (8). We observed that the proliferative response to IFX increased before the second cycle, but became negative at the following administrations, while the response to Phl p5 was consistently positive in all samples (Fig 2A). In agreement with the timing of T cell proliferation, we found that drug-specific IFN- γ -producing cells (Elispot assay, Diaclone SAS, Biotech, France) were up-regulated at the second desensitization cycle (Fig 2B). As a whole, these data suggest that desensitization induces an early increase of cellular (as noted for the humoral) response to the drug, followed by a subsequent decrease.

Analyzing the regulatory cytokines (ELISA assay-R&D Systems, Minneapolis, MN for TGF- β ; Affymetrix eBioscience, Vienna, Austria for IL-10 and IL-37; CUSABIO Biotech CO. LTD for IL-35), during the desensitization cycles, we observed unchanged TGF- β and undetectable IL-37 and IL-10 levels (data not shown). By contrast, serum IL-35 was highly increased after each cycle and, importantly, we observed a progressive increase of baseline values, in serum samples collected before each cycle (Fig 2C). Moreover, PBMC upon *in vitro* stimulation with IFX were able to

96 produce IL-35 in a MHC-Class II-restricted manner, suggesting that the production of this
97 regulatory cytokine is sustained by the presence of drug-specific IL-35-producing cells [T
98 regulatory (Tr35) cells] (Fig 2D). It was recently shown that Tr35 cells express check point
99 molecules, including PD1 (9); in our case we observed that the proportion of circulating
100 CD3+CD4+PD1+ and CD3+CD4+Foxp3+ T cells peaked at the second and third desensitization,
101 respectively (Fig 2E-2F).

102 Although there is as yet no consensus in literature about the underlying mechanisms operating in
103 desensitization, the majority of data focused on the role of mast cells and their hyporesponsiveness.
104 Our results provide evidence that, beyond the interference with mast cells function, the
105 desensitization procedure may be able to modulate the adaptive immune response. To our
106 knowledge, this is the first demonstration that during desensitization for a monoclonal antibody, a
107 decrease of both non isotype and IgE-specific anti-drug antibodies may occur. Accordingly, in this
108 patient we had the opportunity to evaluate the effects of desensitization on the humoral and cellular
109 response to the culprit drug vs an unrelated allergen, allowing us to demonstrate that the adaptive
110 immune response is modulated in an antigen-specific manner, thus contributing to the
111 characteristic antigen-specificity of desensitization.

112 Furthermore, the involvement of regulatory mechanisms, such as activation/expansion of drug-
113 specific Tr35 cells, may occur during the procedure and participate in the modulation of effector
114 drug-specific response. Such response to high antigen dose is likely comparable to that described
115 for allergy immunotherapy in which IL-35 has been recently described to play a relevant role (10).
116 In conclusion, this study suggests that desensitization cycles affect cellular and humoral response to
117 the drug much more than it has been described so far, thus contributing to prevent new reactions
118 and to allow the continuation of the therapeutic program.

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FIGURE LEGENDS

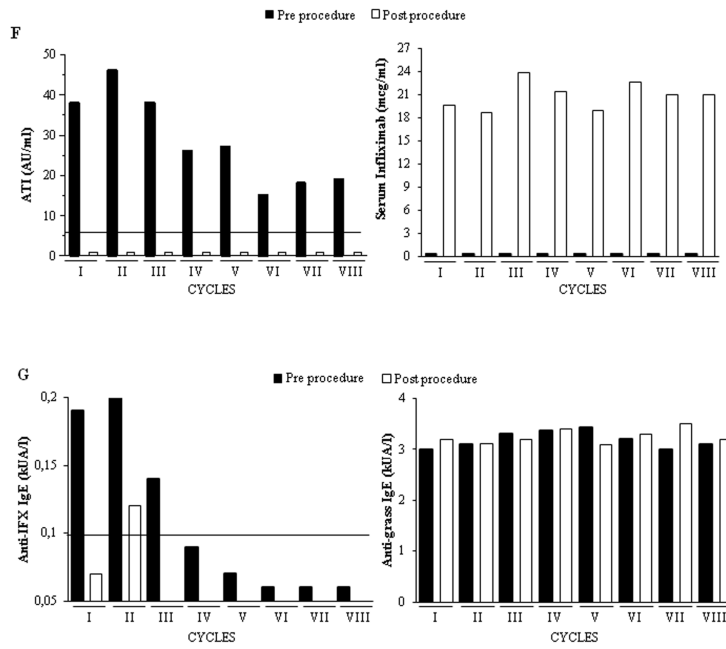
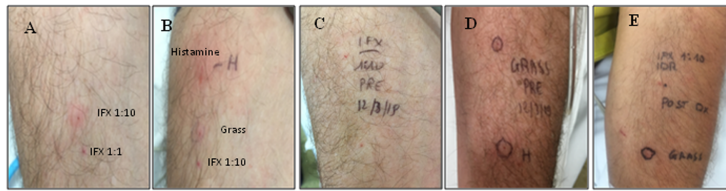
FIG 1. Skin testing for infliximab, grass pollen extract and histamine before (A) and after (B) the first cycle and before (C, D) and after (E) the eighth cycle. F, Monitoring of pre- and post-procedure serum ATI (left panel) and infliximab serum levels (right panel). G, Monitoring of pre- and post-procedure serum anti-infliximab (left panel) and anti-Phl p5 (right panel) IgE antibodies. ATI, anti-infliximab antibodies

FIG 2. A, desensitization effect on PBMC proliferation to infliximab and Phl p5 allergen, restricted to MHC class II elements, evaluated by 3H uptake. B, IFN- γ -producing T cells upon infliximab-specific stimulation. C, Pre- and post-procedure IL-35 serum levels during desensitization. D, IL-35 production by infliximab-specific PBMC. E, Percentage of CD3+CD4+Foxp3+ (■) and CD3+CD4+PD1+ (□) cells evaluated before the first five cycles of desensitization by FACS. F, FACS plot show the quantitative change in CD25+PD1+ (CD3+CD4+ gated) cells before and after the first desensitization. PBMC, peripheral blood mononuclear cells; IFX, infliximab; FACS, fluorescence-activated cell sorting.

Table E1. 16-step desensitization protocol to Infliximab

Step	Solution	Rate (ml/h)	Time (min)	Volume infused (ml)/step	Dose administered (mg)/step	Cumulative dose (mg)
1	1	2,5	15	0,625	0,00145	0,0025
2	1	5	15	1,25	0,0029	0,0054
3	1	10	15	2,5	0,0058	0,0112
4	1	20	15	5	0,0116	0,0228
5	2	2,5	15	0,625	0,0145	0,0373
6	2	5	15	1,25	0,029	0,0663
7	2	10	15	2,5	0,058	0,1243
8	2	20	15	5	0,116	0,2403
9	3	5	15	1,25	0,29	0,5303
10	3	10	15	2,5	0,58	1,1103
11	3	20	15	5	1,16	2,2703
12	3	40	15	10	2,32	4,5903
13	4	10	15	2,5	5,8	10,3903
14	4	20	15	5	11,6	21,9903
15	4	40	15	10	23,2	45,1903
16	4	80	174,375	232,5	534,8097	580

From Bonamichi-Santos R et al ²



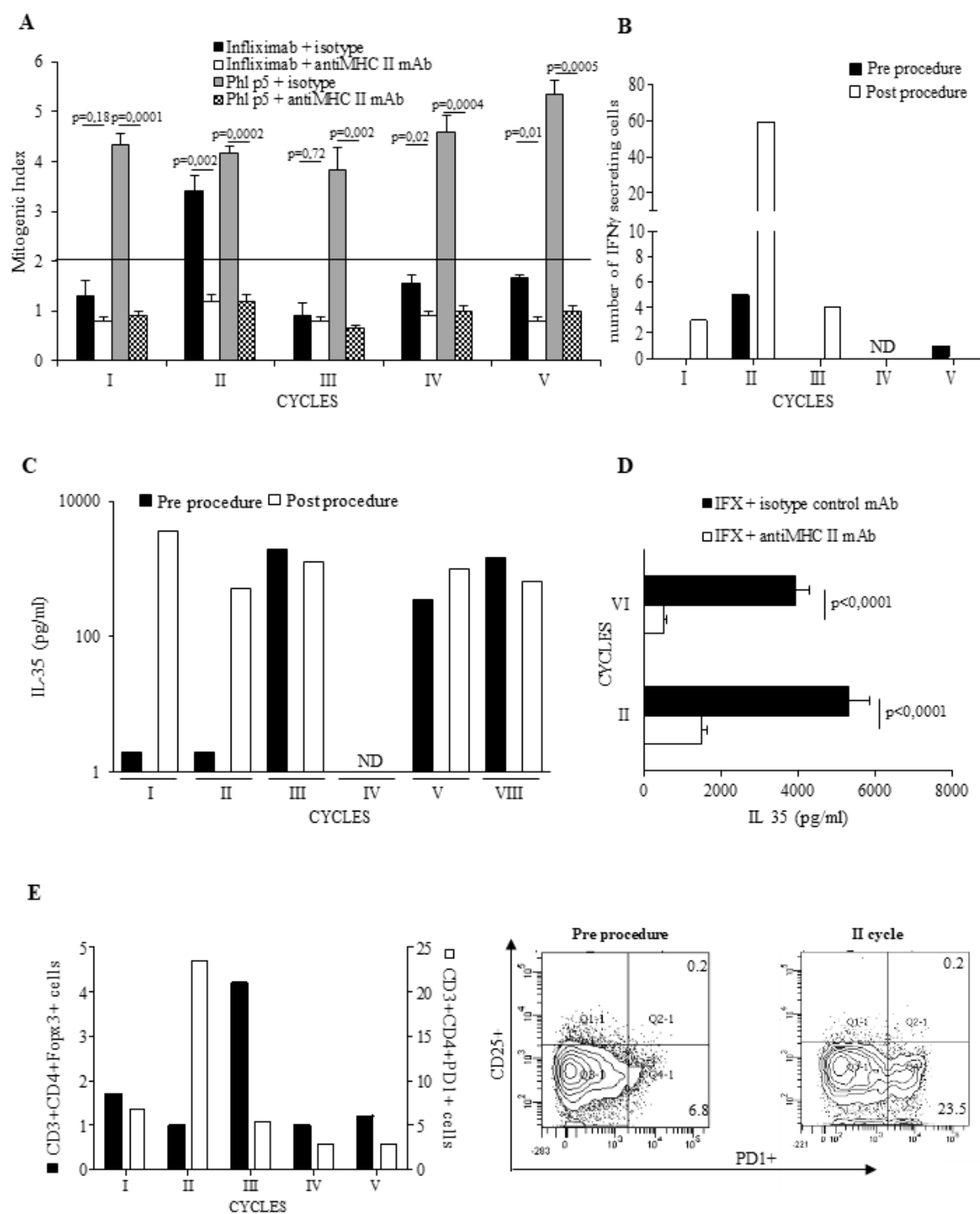


Figure 2