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RESEARCH LETTER

IL-2 antibodies in type 1 diabetes and during IL-2 therapy

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Abbreviations

aAbs	Autoantibodies
AU	Arbitrary units
ld-IL-2	Low-dose IL-2
hd-IL-2	High-dose IL-2
rhIL-2	Recombinant human IL-2

To the Editor: Natural autoantibodies (aAbs) targeting cytokines, chemokines and growth factors have been described in healthy individuals [1], usually at very low levels. It has been proposed that such aAbs may regulate cytokine homeostasis and function by different mechanisms [1]: (1) cytokine neutralisation, (2) as cytokine–aAb complexes, prolongation of the half-life of the cytokines, and (3) signalling via the Fc receptor. Numerous studies have reported highly inconsistent prevalence of anti-IL-2 aAbs in healthy individuals (from 0% to 100%) [2, 3] and in individuals with various diseases [2, 3]. There are as yet no reported clinical consequences associated with anti-IL-2 aAbs. Many studies have reported induction of anti-IL-2 aAbs in individuals receiving high-dose IL-2 (hd-IL-2), without evidence of a clinical impact [4].

Recently, Pérol et al [5] reported anti-IL-2 aAbs in NOD mice and in approximately 20% of individuals with type 1 diabetes using a solid-phase assay. Given the central role of IL-2 in immune tolerance [6], Pérol et al proposed that anti-IL-2 aAbs could impair immune tolerance and play a causal role in type 1 diabetes. However, these results have been recently challenged. Using a liquid-phase detection assay rather than IL-2-coated plates, Marzinotto et al [7] did not find any differences in the anti-IL-2 reactivity of individuals with or without type 1 diabetes, and concluded that the results 'do not support the presence of IL-2 autoantibodies or their relevance for the pathogenesis of human type 1 diabetes' [7].

To further address the existence and relevance of anti-IL-2 aAbs in type 1 diabetes, we analysed sera from individuals with type 1 diabetes and various controls, including participants treated with low-dose IL-2 (ld-IL-2) (written informed consent was obtained before enrolment and clinical trials were conducted in accordance with the Declaration of Helsinki). We previously reported that we did not detect anti-IL-2 aAbs in individuals with type 1 diabetes who were enrolled in our ld-IL-2 trial (DF-IL2 [8], NCT01353833) when measuring total immunoglobulins [9]. As Pérol et al [5] measured anti-

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IgG antibodies, we re-tested our type 1 diabetic individuals using the same ELISA as Pérol et al [5], which he had initially developed in our laboratory. Briefly, 96-well plates (Medisorp, Nunc, Waltham, MA, USA) were incubated overnight with recombinant human IL-2 (rhIL-2; Proleukin, Novartis, Basel, Switzerland), following which plates were blocked and then incubated with serially diluted serum samples in duplicate. Biotin-labelled anti-human total IgG (1:5000; Southern Biotech, Birmingham, AL, USA) was used as the primary antibody, followed by incubation with horseradish peroxidase (HRP)-conjugated streptavidin (1:2000; Invitrogen, Carlsbad, CA, USA). Absorbance was read at 450 nm with a DTX 880 Multimode Detector (Beckman Coulter, Brea, CA, USA).

Our analysis included healthy individuals and individuals treated with ld-IL-2 for hepatitis C virus-induced cryoglobulinemia vasculitis (Vascu-IL2, NCT00574652 [10]), or treated with hd-IL-2 for primary refractory or relapsed neuroblastoma (NCT01701479). All statistical analyses were performed using GraphPad Prism v6 (GraphPad Software, CA, USA). A p value of <0.05 was considered significant. Data presented here are additional measurements performed on sera collected by the investigators during the clinical trials.

At baseline, anti-IL-2 IgG were (mean ± SEM) 30.8 ± 7.9 arbitrary units (AU) for the healthy individuals, 57.2 ± 5.4 AU for type 1 diabetes individuals ($p = 0.0005$ vs healthy individuals, Mann–Whitney unpaired test), 22.2 ± 4.3 AU for individuals with vasculitis (p = NS vs healthy individuals), and 24.9 ± 8.3 AU for individuals with neuroblastoma (p = NS vs healthy individuals) (Fig. 1a). Thus, serum reactivity against IL-2-coated plates in individuals with type 1 diabetes is approximately twofold that in healthy people or in individuals with vasculitis or neuroblastoma. However, this baseline reactivity is approximately 60 times lower than the reactivity generated after treatment with hd-IL-2 in some individuals with neuroblastoma (Fig. 1a). Pérol et al [5] proposed that the anti-IL-2 reactivity in individuals with type 1 diabetes could contribute to disease pathophysiology by reducing IL-2 availability and impacting regulatory T cell (Treg) fitness. Thus, we investigated the correlation between anti-IL-2 aAbs and proportions and activation status of Tregs [9]. Using the non-parametric Spearman correlation test, we found no correlation between anti-IL-2 IgG levels and (1) Treg proportions (Fig. 1b) or (2) Treg CD25 expression (Fig. 1c) at baseline. Pérol at al [5] also noted an association between anti-IL-2 reactivity and age in type 1 diabetic individuals. We did not find a correlation between anti-IL-2 reactivity and duration of disease (Fig. 1d) or age (Fig. 1e) in our type 1 diabetic individuals. Taken together, our results indicate low serum reactivity against IL-2-coated plates in some individuals with type 1 diabetes. In the absence of any consequence of this reactivity, which is not found against soluble IL-2 [7], this finding remains of unclear significance and may be an artefact.

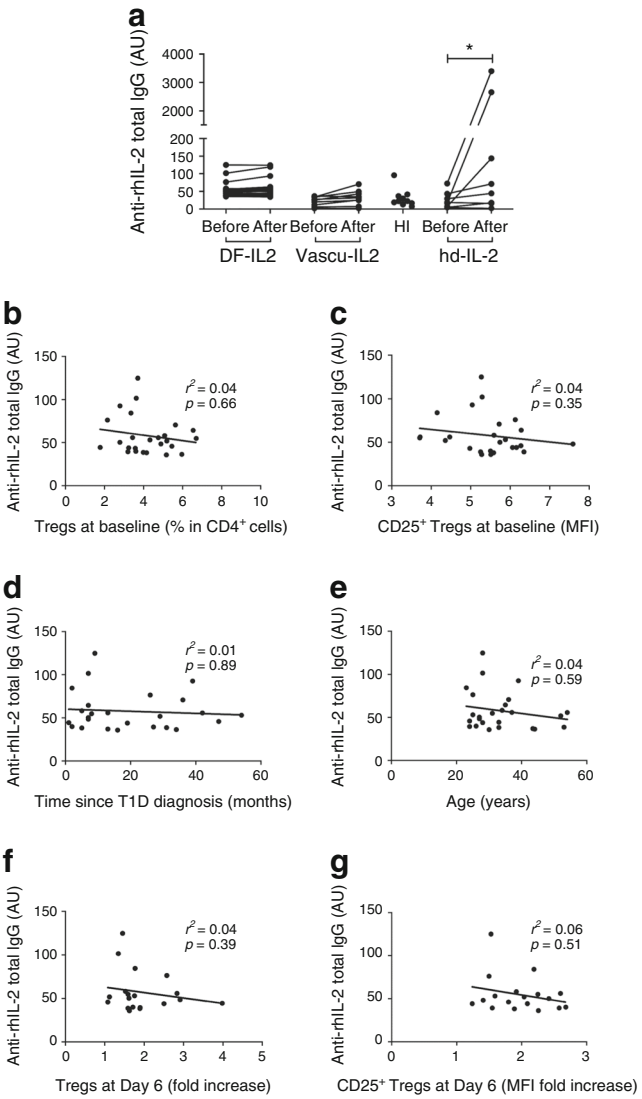

Fig. 1 Anti-IL-2 antibody study. Participants' sera were tested for the presence of anti-IL-2 antibodies before and after IL-2 treatment and compared with sera from various controls. (**a**) Individual evolution of anti-rhIL-2 IgG in participants (DF-IL2: $n = 19$; Vascu-IL2: $n = 9$; neuroblastoma hd-IL-2: $n = 8$) and healthy individuals (HI: $n = 10$). (**b**–**g**) Correlation between anti-rhIL-2 IgG at baseline in DF-IL2 participants and (**b**) percentage of Tregs before treatment ($n = 25$), (**c**) CD25$^+$ Tregs before treatment ($n = 25$), (**d**) time since type 1 diabetes diagnosis ($n = 25$), (**e**) age ($n = 25$), (**f**) increase in Tregs after five consecutive daily injections of IL-2 ($n = 19$), and (**g**) increase in CD25$^+$ Tregs after five consecutive daily injections of IL-2 ($n = 19$). AU, arbitrary units; MFI, mean fluorescence intensity

Given the central role of Tregs in the pathogenesis of autoimmune diseases, and the capacity of ld-IL-2 to specifically expand and activate Tregs, ld-IL-2 is being evaluated as an immunoregulatory treatment for several autoimmune diseases [6], including type 1 diabetes. Pérol et al [5] reported circulating IL-2 specific memory B cells in type 1 diabetic individuals and suggested that they could be boosted by ld-IL-2

(Proleukin, rhIL-2, Novartis) administration and affect the response of Tregs to IL-2. We addressed this important question in our trial participants treated with IL-2.

We did not observe any boosting of the anti-IL-2 serum reactivity of type 1 diabetic individuals sera after ld-IL-2 administration (Fig. 1a). The post-treatment anti-IL-2 reactivity of these participants (58.7 ± 6.3 AU) and that of treated individuals with vasculitis (32.6 ± 6.9 AU) was not significantly different from baseline (p = NS, Wilcoxon paired test). In contrast, we observed an increased reactivity in two individuals with neuroblastoma treated with hd-IL-2 (Fig. 1a). We also investigated whether levels of anti-IL-2 reactivity at baseline could affect the Treg response to ld-IL-2 treatment. There was no correlation between anti-IL-2 reactivity at baseline and the increase (1) in the proportion of Tregs (Fig. 1f) or (2) in Treg CD25 expression (Fig. 1g) after five consecutive daily ld-IL-2 injections.

Overall, our current data, based on a short course of ld-IL-2 therapy, do not support a direct role for anti-IL-2 reactivity in type 1 diabetes pathogenesis via effects on Tregs, or that anti-IL-2 reactivity could be a major pitfall of ld-IL-2 therapy. It is possible that anti-IL-2 antibody production may be induced following prolonged courses of ld-IL-2 administration. However, to date, we have not detected anti-IL-2 antibodies in our TRANSREG (NCT01988506) or our DFIL2-child (NCT01862120) clinical trials, in which 69 and 24 participants received ld-IL-2 for 6 and 12 months, respectively (D. Klatzmann, unpublished results). Finally, it remains to be shown that the reactivity to IL-2-coated plates found in type 1 diabetic individuals is caused by bona fide anti-IL-2 aAbs, i.e. dependent on a specific interaction of the antibody variable regions with IL-2. We suggest that until this is demonstrated, it would be prudent to refer to these findings as reflecting an 'anti-IL-2 reactivity' rather than anti-IL-2 aAbs.

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Data availability Data are available from the authors upon request.

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Duality of interest MR, PC, DS and DK are inventors on a patent application related to the therapeutic use of ld-IL-2, which belongs to their academic institutions and has been licensed to ILTOO Pharma. MR, PC, GC and DK hold shares in ILTOO Pharma.

Contribution statement PC, DS and DV performed the participants' follow-up. GC, MR and NC conducted the experiments. All authors analysed the data. GC, AP and DK wrote the manuscript, and all authors revised and approved the manuscript. DK and GC are responsible for the integrity of the work as a whole.

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