EBV infection and anti-CD3 treatment for Type 1 diabetes: bad cop, good cop?

Expert Rev. Clin. Immunol. 9(2), 95–97 (2013)

Denise L Faustman

Immunobiology Labs, Massachusetts General Hospital and Harvard Medical School, Room 3602, MGH-East, Boston, MA 02129, USA
faustman@helix.mgh.harvard.edu

"Closer examination of anti-CD3 clinical trials points to EBV reactivation as a possible contributor to efficacy."

Two high-profile Phase III clinical trials of the immunosuppressant anti-CD3 monoclonal antibody returned disappointing results for patients with Type 1 diabetes (T1D) [1,101]. Anti-CD3 had looked highly promising based on its excellent performance in Phase II clinical trials [2,3]. What went wrong? One explanation may be that the dose of anti-CD3, which had been lowered for the Phase III trials, was insufficient to reactivate patients' prior EBV infection. EBV reactivation is a well-established side effect of immunosuppression. EBV reactivation, which had occurred in the Phase II clinical trials, was likely to have been one of the motivations for reducing the dose of anti-CD3 in the Phase III clinical trials. However, instead of being an unwanted side effect, EBV may have paradoxically contributed to the efficacy of anti-CD3 in the higher-dose Phase II trials. EBV reactivation, in other words, may be a desirable side effect as it relates to tumor necrosis factor (TNF) induction.

A beneficial role for EBV comes from understanding its pathophysiology and influence on the immune system. It has been known for decades that EBV, as part of the innate host response, is a potent inducer of host TNF. Some of the regulatory immune function of anti-CD3 requires TNF [4]. TNF has been known in animal models to suppress or prevent onset of T1D by selectively destroying insulin-autoreactive T cells and inducing beneficial Treg cells, according to diverse evidence [5–7]. In a newly published randomized controlled clinical trial for T1D, acute EBV infection by itself, in the absence of any immunosuppressive treatment, induces TNF and leads to these salutary effects: death of insulin-autoreactive T cells that attack and destroy islets, induction of beneficial Treg cells and a transient restoration of insulin secretion assessed by C-peptide [8]. The clinical trial was designed to test the efficacy of the nonvirulent microbe BCG, another known inducer of host TNF. Serendipitously, a placebo patient in the trial had an acute, undiagnosed case of EBV infection at baseline. The patient completed the 5-month trial and was subjected to the same analyses as other patients. The placebo patient had the same robust responses against T1D as did the BCG-treated patients. The reason for the success is most likely because both microbes, EBV and BCG, induce release of host TNF [8].

Closer examination of anti-CD3 clinical trials points to EBV reactivation as a possible contributor to efficacy. Using high doses of anti-CD3 antibody (34–48 mg/total dosing/70 kg), a Phase II clinical trial found that 30 of 40 treated diabetic subjects exhibited reactivation of EBV, manifested by EBV viral load and mono-like symptoms with onset 16–21 days after first administration of antibody [2]. This short-term, high-dose anti-CD3 antibody treatment preserved residual pancreas function in new onset T1D, as measured by C-peptide. Systemic TNF induction occurred within days of anti-CD3 administration [2], an effect that could be attributable to both the EBV reactivation and the anti-CD3 antibody.

Keywords: anti-CD3 • BCG • Epstein–Barr virus • tumor necrosis factor • Type 1 diabetes

EXPERT REVIEWS

 10.1586/ECI.12.101 ISSN 1744-666X

In another high-dose Phase II anti-CD3 trial, a follow-up laboratory study found that monitored patients with acute elevations in EBV-reactive T cells also exhibited release into the circulation of EBV-reactive antibodies and autoreactive T cells [9]. Similarly, the BCG clinical trial found a rapid rise of autoreactive T cells days after onset of an acute EBV infection without anti-CD3 administration. By flow cytometry, the autoreactive T cells were mostly dead [8]. When dosing of anti-CD3 results in EBV reactivation, the combined induction of TNF may facilitate the death of autoreactive T cells. This effect was observed in the BCG clinical trial with the EBV placebo patient and the BCG-treated patients.

The mechanism by which TNF selectively kills insulin-autoreactive T cells is known to involve signaling defects in the TNF pathway in humans with T1D [5,7]. More specifically, in the nonobese diabetic (NOD) animal model of T1D and Sjogren's syndrome, autoreactive T cells are selectively vulnerable to cell death in the TNF pathway because of abnormal proteasomes in lymphoid cells [10–14]. Normal proteasomes, upon TNF exposure, activate the transcription factor NF-κB, which then translocates to the nucleus to trigger expression of prosurvival genes. With an abnormal proteasome, NF-κB activation by TNF is blocked, precluding expression of prosurvival genes, thereby leading to cell death. Normal T cells, unlike autoreactive T cells, are not vulnerable to TNF-induced death because they constitutively express NFκB; they do not rely on intact proteasomes for TNF intracellular signaling and expression of prosurvival genes. TNF signaling is disrupted in other ways in several autoimmune diseases [15].

The two unsuccessful Phase III clinical trials of anti-CD3 saw the near complete absence of EBV reactivation, defined as lacking mono-like symptoms and viral loads [1,101]. Both trials failed to meet their prespecified clinical end points. One anti-CD3 clinical trial (otelixizumab) utilized a 15-fold reduced dosage of anti-CD3 antibody, from a cumulative total of 48 mg in the Phase II trial down to 3.1 mg [101]. At this extremely low dose, anti-CD3 demonstrated no EBV reactivation by viral loads, nor TNF induction and no C-peptide maintenance. The other Phase III trial of anti-CD3 (teplizumab) administered cumulative doses of 4.6–17 mg, with the high dose similar to the Phase II dose level [1]. Because 85% of treated subjects versus less than 10% of placebo group had past EBV infections, the study may have been biased to find an effect in treated subjects as it relates to EBV or at least the possible beneficial effect of EBV cannot be evaluated. Even though this trial did not meet its primary efficacy end point, there was less decline of C-peptide in the high-dose anti-CD3 group compared with placebo, and these subjects almost exclusively had past EBV infections unlike the placebo group.

Taken together, the human clinical trials, as well as the mechanistic studies, suggest that EBV reactivation, through induction of TNF, may contribute to the efficacy of anti-CD3 antibodies. This novel mechanism, which harnesses innate immunity, may be ripe for cultivation with existing or new immunosuppressive agents. The anti-CD3 monoclonal clinical trials and the BCG clinical trial, which monitored acute EBV infection, show the benefit of infections that induce innate immunity by triggering release of host TNF [16]. This point is consistent with the decades-old hygiene hypothesis, which attributes the rise in autoimmunity and allergies to the removal of infections in modern societies [17]. Reintroduction of infections after T1D onset, perhaps with an attenuated EBV for example, may help to restore immune balance.

Acknowledgements

Proofreading and editorial assistance provided by Miriam Davis of our department.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Sherry N, Hagopian W, Ludvigsson J *et al.*; Protégé Trial Investigators. Teplizumab for treatment of Type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 378(9790), 487–497 (2011).

2 Keymeulen B, Vandemeulebroucke E, Ziegler AG *et al.* Insulin needs after CD3-antibody therapy in new-onset Type 1 diabetes. *N. Engl. J. Med.* 352(25), 2598–2608 (2005).

3 Herold KC, Hagopian W, Auger JA *et al.* Anti-CD3 monoclonal antibody in new-onset Type 1 diabetes mellitus. *N. Engl. J. Med.* 346(22), 1692–1698 (2002).

4 Ablamunits V, Bisikirska B, Herold KC. Acquisition of regulatory function by human CD8(+) T cells treated with anti-CD3 antibody requires TNF. *Eur. J. Immunol.* 40(10), 2891–2901 (2010).

5 Faustman D, Davis M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat. Rev. Drug Discov.* 9(6), 482–493 (2010).

6 Dale E, Davis M, Faustman DL. A role for transcription factor NF-kappaB in autoimmunity: possible interactions of genes, sex, and the immune response. *Adv. Physiol. Educ.* 30(4), 152–158 (2006).

7 Ban L, Zhang J, Wang L, Kuhtreiber W, Burger D, Faustman DL. Selective death of autoreactive T cells in human diabetes by TNF or TNF receptor 2 agonism. *Proc. Natl. Acad. Sci. U.S.A.* 105(36), 13644–13649 (2008).

8 Faustman DL, Wang L, Okubo Y *et al.* Proof-of-concept, randomized, controlled clinical trial of Bacillus Calmette–Guérin for treatment of long-term Type 1 diabetes. *PLoS One* 7(8), e41756 (2012).

9 Cernea S, Herold KC. Monitoring of antigen-specific CD8 T cells in patients with Type 1 diabetes treated with antiCD3 monoclonal antibodies. *Clin. Immunol.* 134(2), 121–129 (2010).

10 Yan G, Fu Y, Faustman DL. Reduced expression of Tap1 and Lmp2 antigen-processing genes in the nonobese diabetic (NOD) mouse due to a mutation in their shared bidirectional promoter. *J. Immunol.* 159(6), 3068–3080 (1997).

11 Fu Y, Yan G, Shi L, Faustman D. Antigen processing and autoimmunity. Evaluation of

mRNA abundance and function of HLA-linked genes. *Ann. N. Y. Acad. Sci.* 842, 138–155 (1998).

12 Hayashi T, Faustman D. NOD mice are defective in proteasome production and activation of NF-kappaB. *Mol. Cell. Biol.* 19(12), 8646–8659 (1999).

13 Hayashi T, Kodama S, Faustman DL. Reply to 'LMP2 expression and proteasome activity in NOD mice'. *Nat. Med.* 6(10), 1065–1066 (2000).

14 Krause S, Kuckelkorn U, Dörner T, Burmester GR, Feist E, Kloetzel PM. Immunoproteasome subunit LMP2 expression is deregulated in Sjogren's syndrome but not in other autoimmune disorders. *Ann. Rheum. Dis.* 65(8), 1021–1027 (2006).

15 Gregori G, Ravasio A, Murphy CD *et al.* Generation of scaled protogalactic seed magnetic fields in laser-produced shock waves. *Nature* 481(7382), 480–483 (2012).

16 Rahman MM, McFadden G. Modulation of tumor necrosis factor by microbial pathogens. *PLoS Pathog.* 2(2), e4 (2006).

17 Strachan DP. Hay-fever, hygiene, and household size. *Br. Med. J.* 299(6710), 1259–1260 (1989).

Website

101 GlaxoSmithKline and Tolerx announce Phase III DEFEND-1 study of otelixizumab in Type 1 diabetes did not meet its primary endpoint. GlaxoSmithKline press release (2011). http://us.gsk.com/html/media-news/pressreleases/2011/2011_pressrelease_10039.htm