Diabetologia (2019) 62:1727–1729
https://doi.org/10.1007/s00125-019-4893-2

LETTER

CD40-targeted peptide proposed for type 1 diabetes therapy lacks relevant binding affinity to its cognate receptor

Philippe P. Pagni[1] · Anitra Wolf[1] · Mauro Lo Conte[1] · Ronald Yeh[1] · Guangsen Fu[1] · Fa Liu[1] · Matthias von Herrath[1] · Ken Coppieters[2]

Received: 29 January 2019 / Accepted: 3 April 2019 / Published online: 17 May 2019

Keywords CD40 · CD40L · Type 1 diabetes

Abbreviations

CD40L	CD40 ligand
SPR	Surface plasmon resonance

To the Editor: The CD40-CD40 ligand (CD40L, also known as CD154) pathway has been identified as a potential target for pharmaceutical intervention in type 1 diabetes [1, 2]. The CD40-targeted peptide approach published by Wagner and colleagues in *Diabetologia* in 2014 [3] captured our attention because their data indicated that the peptide used in their research induced potent diabetes prevention and possible diabetes reversal in the gold-standard NOD mouse model of type 1 diabetes. Moreover, in contrast to other anti-CD40L antibodies, it was suggested that the peptide format used in the paper by Wagner's group [3] would not cause anti-CD40L immune complexes to trigger Fcγ type 2 receptor A (FcγRIIa) signalling through an Fc moiety, thus avoiding platelet activation and the subsequent thrombotic complications associated with these antibodies [4].

The in vivo data obtained by Wagner's group [3] imply that the CD40L-derived 15-mer peptide would bind to CD40 with high enough affinity to block endogenous CD40L binding or to induce any other sort of uncharacterised tolerogenic signalling downstream of CD40.

To assess 15-mer peptide binding to CD40, we first produced the mouse 15-mer peptide and a scrambled 15-mer version, confirmed solubility by visual inspection and verified identity by mass spectroscopy (MS; Fig. 1a) and purity via C18 reversed phase HPLC (data not shown) (see ESM Methods for further details). We proceeded to characterise the peptide's binding association and dissociation kinetics to mouse CD40 via biolayer interferometry using an Octet Red instrument (Fremont, CA, USA) (Fig. 1b). The commercial control mouse CD40L protein (R&D Systems; Minneapolis, MN, USA) demonstrated strong affinity for mouse CD40, estimated in the low nmol/l range, with fast on-rate and slow off-rate binding kinetics. In contrast, very fast on and fast off kinetics were observed for both the 15-mer peptide and the scrambled version of the peptide, possibly indicating non-specific binding. To evaluate binding using an alternative method, surface plasmon resonance (SPR) was employed to quantitatively determine the affinity of 15-mer binding to mouse CD40-Fc (see ESM Methods). The commercial CD40L protein bound to CD40 with a binding affinity (K_D) of 0.4 nmol/l (Fig. 1c), but no binding was seen using up to 3 μmol/l of the 15-mer (Fig. 1d) or scrambled peptides (data not shown).

Next, we performed structural analysis by aligning the peptide with the published CD40-CD40L crystal structure in silico in order to evaluate binding location and theoretical interaction quality (see ESM Methods) [5]. This analysis was performed under the assumption that synthesised, free and flexible 15-mer peptide will retain the same conformation as native CD40L when interacting with CD40. It was determined that peptide interaction with CD40 is mediated by residues K143, G144, Y145 and Y146 (hydrophilic contacts), and also E142 and K143 (charge interactions) [5]. George Jeffrey categorised H bonds with donor-acceptor distances of 3.2–4.0 Å as weak and electrostatic; the 15-mer peptide CD40 interaction region was predicted to have H bonds that fell within these donor-accepted distances (see Fig. 1e), [6].

✉ Ken Coppieters
kncp@novonordisk.com

[1] Novo Nordisk Research Center, Seattle, WA, USA

[2] Novo Nordisk A/S Research Projects, Novo Nordisk Park, DK-2760 Måløv, Denmark

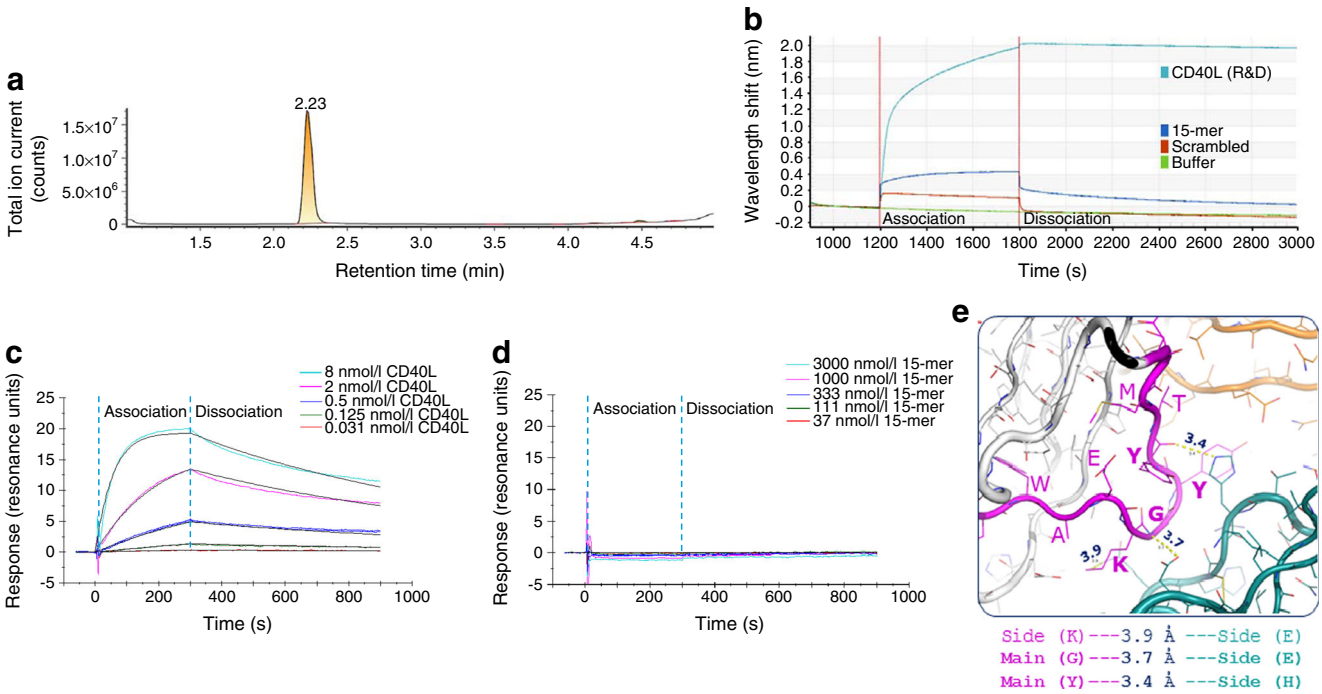

Fig. 1 Experimental and in silico assessment of the properties of 15-mer peptide binding to CD40. (**a**) Liquid chromatography–mass spectrometry (LC–MS) verification of the 15-mer peptide batch used in binding experiments. Observed Mass (Da) was 1816.9798. (**b**) Octet Red assay data on 15-mer and scrambled 15-mer peptides vs commercial CD40L (R&D Systems). (**c**) Analysis of the binding of CD40L to mouse CD40-Fc by SPR. CD40-Fc was captured on a chip surface immobilised with goat anti-human Fc antibody. Then, CD40L was injected over that surface at different concentrations for 300 s followed by a dissociation step, which involved buffer flowing over the chip for 600 s. The calculated binding affinity (K_D, defined by the ratio of binding rate constants k_d/k_a) was determined as 0.4 nmol/l. (**d**) Analysis of the binding of the CD40-targeted 15-mer peptide to mouse CD40-Fc by SPR. As in (**c**), the CD40-targeted 15-mer peptide was injected at different concentrations over the same chip that captured CD40-Fc. No binding was observed up to 3 μmol/l (3000 nmol/l). (**e**) Modelled interaction region of relevant interaction residues between the 15-mer peptide (magenta) and CD40 (green). The interacting residues from the peptide are shown in bold

We conclude that, according to the analysis based on the crystal structure of CD40/CD40L, the 15-mer peptide's interface with CD40 is very limited and primarily relies on main chain interactions. In selecting a CD40-binding peptide from the CD40L sequence, solely based on the published complex structure, other regions of CD40L display better properties than this 15-mer in terms of binding interface. However, the 15-mer peptide would be flexible in solution and most probably could adopt a totally different conformation compared with its conformation in the complex structure.

Taken together, our data do not support the hypothesis that the 15-mer CD40-targeted peptide published by Wagner and co-authors [3] is capable of establishing any relevant binding interaction with CD40. The authors claimed to have been able to pull down native CD40 protein from a complex immune cell lysate using beads coated with 15-mer peptide. In addition, a fluorescently labelled 15-mer peptide was reported to stain immune cells in flow cytometry analysis. Based on the data presented here, we propose that binding may have occurred due to a random endogenous protein in an off-target, non-CD40 specific fashion. As for the NOD mouse model data, we noted that the efficacy of this peptide in preventing hyperglycaemia was obtained using a once weekly i.v. dosing regimen, beginning at 5 weeks of age. With an expected half-life in the order of minutes [7] and virtually no affinity for CD40, we consider the probability of meaningfully affecting the CD40–CD40L pathway with the 15-mer peptide to be extremely low. It is, of course, possible that an uncharacterised off-target mechanism is affected by the 15-mer peptide that could result in the efficacy profile observed, such as that in the early prevention setting in the NOD model. The data from the recent-onset diabetes model published by Wagner's group [3], on the other hand, suggest reversal in $n = 5$ mice vs no reversal in $n = 4$ mice, which alone remains an inconclusive observation.

In conclusion, our dataset casts doubt on the findings published by Wagner's group [3] and question the rationale for progression of the 15-mer peptide used in their studies towards use in clinical studies. Wagner and colleagues concluded that their alternative therapeutic peptide approach opens new avenues of exploration in targeting receptor–ligand interactions [3]. Based on our data, we conclude that, when isolating small peptides from their native context within the receptor binding region, it is rather uncertain that any relevant binding affinity will be preserved or therapeutic utility achieved.

Data availability All data are available on request from the authors.

Funding All authors performed this work while under employment of Novo Nordisk.

Duality of interest The authors declare employment at Novo Nordisk. There is no duality of interest associated with this manuscript.

Contribution statement PPP, AW, MLC, RY, GF and FL contributed to the conception and design, acquisition of data, or analysis and interpretation of data. MvH and KC contributed to the conception and design and interpretation of data. All authors contributed to drafting the article or revising it critically for important intellectual content and approved of the version to be published. KC is responsible for the integrity of the work as a whole.

References

1. Balasa B, Krahl T, Patstone G et al (1997) CD40 ligand-CD40 interactions are necessary for the initiation of insulitis and diabetes in nonobese diabetic mice. J Immunol 159:4620–4627
2. Homann D, Jahreis A, Wolfe T et al (2002) CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. Immunity 16(3):403–415. https://doi.org/10.1016/S1074-7613(02)00290-X
3. Vaitaitis GM, Olmstead MH, Waid DM, Carter JR, Wagner DH Jr (2014) A CD40-targeted peptide controls and reverses type 1 diabetes in NOD mice. Diabetologia 57(11):2366–2373. https://doi.org/10.1007/s00125-014-3342-5
4. Robles-Carrillo L, Meyer T, Hatfield M et al (2010) Anti-CD40L immune complexes potently activate platelets in vitro and cause thrombosis in FCGR2A transgenic mice. J Immunol 185(3):1577–1583. https://doi.org/10.4049/jimmunol.0903888
5. An HJ, Kim YJ, Song DH et al (2011) Crystallographic and mutational analysis of the CD40-CD154 complex and its implications for receptor activation. J Biol Chem 286(13):11226–11235. https://doi.org/10.1074/jbc.M110.208215
6. Jeffrey GA (1997) An introduction to hydrogen bonding. Oxford University Press, New York
7. Mathur D, Singh S, Mehta A, Agrawal P, Raghava GPS (2018) In silico approaches for predicting the half-life of natural and modified peptides in blood. PLoS One 13(6):e0196829. https://doi.org/10.1371/journal.pone.0196829