ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Chem. Commun.*, 2019, **55**, 253

Received 25th September 2018,
Accepted 3rd December 2018

DOI: 10.1039/c8cc07691j

rsc.li/chemcomm

Published on 03 December 2018. Downloaded by Peking University on 7/7/2020 3:19:44 PM.

β-Glucan as an immune activator and a carrier in the construction of a synthetic MUC1 vaccine†

Hanxuan Wang,[ab] Bing Yang,[ab] Yinglu Wang,[ab] Fen Liu,[a] Alberto Fernández-Tejada *[cd] and Suwei Dong *[ab]

We describe the preparation of a cancer vaccine candidate by conjugating a MUC1 peptide antigen to the β-glucan polysaccharide, which serves both as a carrier and an immune activator. In contrast to amorphous polysaccharides, peptide–β-glucan conjugates form uniform nanoparticles that facilitate the delivery of antigens and binding to myeloid cells, thus leading to the activation of both innate and adaptive immunity.

Vaccination is one of the most significant medical developments in history, which has greatly contributed to the improvement of human health and the increase of the average lifespan.[1] While the early and most currently used vaccines have been developed against infectious diseases, a new and promising direction has emerged to use vaccination in the treatment of cancer.[2,3] As an attractive type of immunotherapy, therapeutic tumour vaccines exploit the power of a patient's own immune system to recognize and kill cancer cells, thereby helping suppress tumour immune escape.[4] Thus, many efforts have been attempted towards the construction of cancer vaccines, including the investigation of suitable carriers,[5–7] tumour specific antigens[8–10] and adjuvants.[11] Among these studies, a carrier protein has usually been employed to deliver the peptide and glycopeptide antigens in a multivalent form, in order to compensate for their lack of adequate stability and immunogenicity. However, the extremely high immunogenicity, as well as the structural complexity and heterogeneity of the carrier protein component, may lead to immunological rejection and associated safety issues. Although a number of carrier protein-free vaccine constructs have been developed, such as those incorporating Toll-like receptor 2 ligands,[12–14] gold nanoparticles,[15] and self-assembling peptides,[16,17] the identification of effective constructs with high immunogenicity is still a challenge that calls for innovative solutions.

β-Glucan is a well-established immune activator that has promising medicinal applications.[18–21] A recent study indicated that β-glucan possesses compelling ability to reverse immune tolerance,[22] thus showing great potential as an immune activator for immunotherapy.[23,24] Mechanistically, β-glucan mainly interacts with the C-type lectin receptor dectin-1, which is widely expressed on the surface of myeloid immune cells such as dendritic cells (DCs), neutrophils, and macrophages, stimulating important immune processes such as antigen presentation, T cell and B cell activation, and cytokine secretion.[25,26] Since dectin-1 is a non-Toll-like receptor, which is a type of innate pattern-recognition receptor (PRR) expressed by myeloid antigen presenting cells (APCs), the activation mode will be distinct in comparison to those of vaccines based on Toll-like receptor (TLR) ligands such as Pam_3Cys-containing vaccines.[27] Besides dectin-1, β-glucan can also bind to other cell surface PRRs including complement receptor 3 (CR3) and the C-type lectin receptor SIGNR1,[28] wherein multiple pathway regulation should be beneficial for immune homeostasis. Herein we report the construction and biological evaluation of a β-glucan-based vaccine, where the carbohydrate component acts as both antigen carrier and immune activator.

The schematic representation of the designed vaccine construct is shown in Fig. 1. The MUC1 tandem repeat sequence GVTSAPDT RPAPGSTPPAH, a well-studied cancer biomarker,[29] was chosen as the peptide antigen to validate our design. An ethylene glycol spacer was used to link β-glucan and the MUC1 peptide. Based on

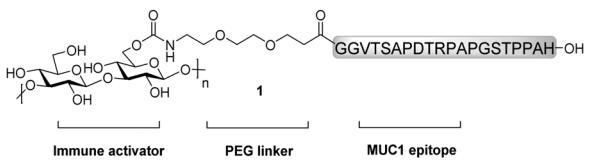


Fig. 1 Design and structure of the β-glucan–MUC1 conjugate based vaccine.

[a] *State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China. E-mail: dongsw@pku.edu.cn*
[b] *Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China*
[c] *Chemical Immunology Laboratory, CIC bioGUNE, Biscay Science and Technology Park, 48160 Derio, Spain. E-mail: afernandeztejada@cicbiogune.es*
[d] *Ikerbasque, Basque Foundation for Science, Maria Díaz de Haro 13, 48009 Bilbao, Spain*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8cc07691j

Published on 03 December 2018. Downloaded by Peking University on 7/7/2020 3:19:44 PM.

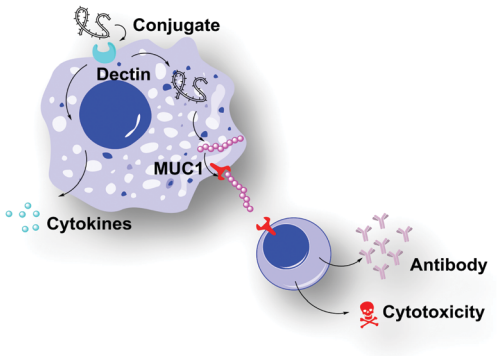
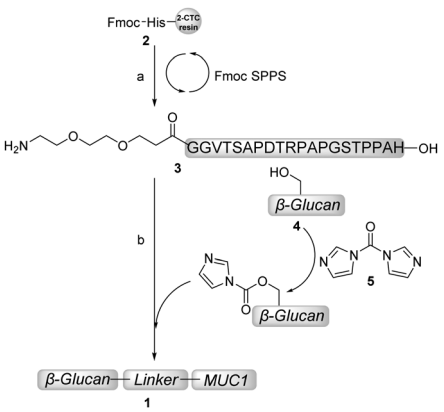


Fig. 2 Proposed mechanism of the conjugate in the process of antigen presentation and immune response elicitation.

the aforementioned immunological roles of β-glucan, we hypothesized that the recognition and uptake of the conjugate by myeloid cells may be able to activate the innate immunity, while the presentation of the MUC1 epitope may synergistically activate the adaptive immunity. Antibodies against the MUC1 peptide antigen will be generated following B cell differentiation into plasma cells, while macrophages, helper T cells, and other immune cells could induce the secretion of various types of cytokines, leading to a more robust immune response (Fig. 2). Such new vaccine constructs that enable dual activation of both innate and adaptive immunity could be particularly interesting models for enhancing the efficacy of cancer immunotherapy.[30]

Our study started with the preparation of the β-glucan–MUC1 peptide conjugate (Scheme 1). Using a typical Fmoc-based solid-phase peptide synthesis (SPPS) strategy, the MUC1 peptide and a PEG linker were sequentially assembled on a 2-chlorotrityl chloride resin (**2**). After global deprotection using a TFA/TIS/H_2O (95 : 2.5 : 2.5, v/v/v) cocktail and purification by HPLC, the desired peptide **3** with a PEG linker was obtained.



Scheme 1 Synthesis of the β-glucan–MUC1 conjugate **1**. (a) Fmoc SPPS: (1) deprotection: piperidine/DBU/DMF (2 : 2 : 96, v/v/v); (2) coupling: Fmoc-AA-OH (4 equiv.) or 9-[(9*H*-fluoren-9-ylmethoxy)carbonylamino]-4,7-dioxanonanoic acid (1.2 equiv.), HATU (4 equiv.), and DIEA (8 equiv.); (3) cleavage: TFA/H_2O/TIPS (95 : 2.5 : 2.5, v/v/v), 15% yield on a 0.02 mmol scale. (b) Synthesis of the conjugate: (1) peptide **3**, β-glucan (**4**), and 1,1′-carbonyl-diimidazole (**5**, 0.5 M in DMSO) (2) Wash with water, dialysis and lyophilisation.

The conjugation reaction with the (1,3)-β-glucan polysaccharide from a yeast extract was performed under 1,1′-carbonyl-diimidazole (CDI)-mediated conditions (see the ESI†). The resulting construct was purified through dialysis to remove the excess peptide, followed by lyophilisation to afford the β-glucan–MUC1 conjugate as a white powder.

To confirm the covalent linkage between the peptide and β-glucan, we first attempted to chemically characterize the conjugate using solution NMR spectroscopy. However, no informative data were obtained due to the poor solubility of β-glucan and its derivative in all the deuterated solvents we tested. Alternatively, ^{13}C solid-state cross polarization magic-angle spinning (CP/MAS) NMR experiments were performed (see the ESI,† page S10), where obvious signals from the peptide were observed in the downfield region (δ 115–145 ppm). Moreover, the signals corresponding to the carbohydrate component were broader in the spectrum of conjugate **1** than those in the spectrum of β-glucan alone. By careful spectral deconvolution, the broad peak at *ca.* 59 ppm was assigned to the combined signals resulted from the C6 of the glucose units with or without peptide derivatization (Fig. S4, ESI†). Using the BCA colorimetric method, the peptide loading was determined to be 7.7 μg of MUC1 antigen per 100 μg conjugate, which was deemed sufficient for the vaccination experiments.

With the vaccine construct in hand, we first explored its biophysical and structural properties using zeta potential measurements, transmission electron microscopy (TEM), and dynamic light scattering (DLS). The zeta potential (Fig. 3a) of the synthetic conjugate showed a higher negative value (−38 mV) than that of β-glucan alone (−23 mV), suggesting that the former is more evenly distributed and has better stability. The morphology of the conjugate and β-glucan was characterized using TEM, which revealed the size of the β-glucan–MUC1 nanoparticles to be in the range of 150 nm (Fig. 3c) while the morphology of β-glucan was found to be irregular (Fig. 3d).

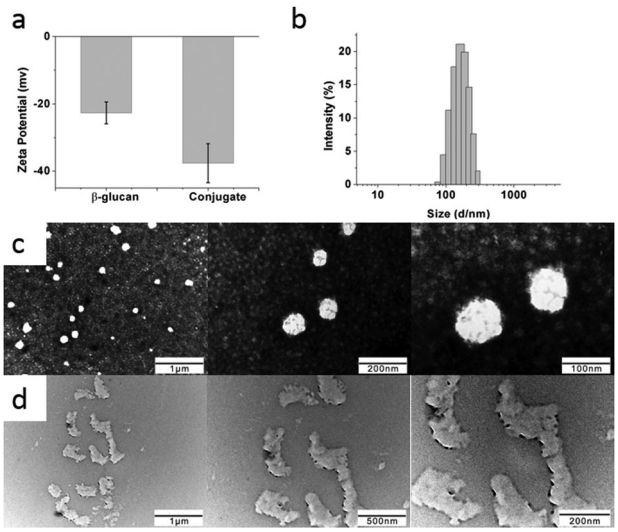


Fig. 3 Characterization of the β-glucan–MUC1 conjugate: (a) zeta potential from dynamic light scattering (DLS) measurements; (b) size distribution from DLS measurements; (c) TEM image of the conjugate; (d) TEM image of untreated β-glucan.

 This journal is © The Royal Society of Chemistry 2019

Published on 03 December 2018. Downloaded by Peking University on 7/7/2020 3:19:44 PM.

Table 1 Data from the DLS experiments

Construct	Diameter (nm)	PDI
β-Glucan	534	0.80
Conjugate	162	0.30

The DLS experiments also revealed a uniform size distribution (intensity) of the conjugate nanoparticles (Table 1 and Fig. 3b), further confirming that the attached peptides increase the stability and homogeneity of the β-glucan nanoparticles.

Next, we assessed the efficacy of the β-glucan–MUC1 conjugate by performing an immunological evaluation in mice. Groups of five C57BL/6 mice were vaccinated subcutaneously with the synthetic conjugate, with a mixture of the MUC1 peptide and β-glucan, with the MUC1 peptide only, with β-glucan only and with PBS buffer, respectively. After four immunizations at weekly intervals (days 0, 7, 14, 21), mouse sera were collected on day 28 and analyzed for the production of anti-MUC1 IgG antibodies. As assessed by the enzyme-linked immunosorbent assay (ELISA), mice vaccinated with the β-glucan–MUC1 conjugate elicited high titers of anti-MUC1 IgG antibodies (Fig. 4a and Fig. S8, ESI†), significantly higher (up to 13 fold) compared to the other control groups (Fig. 4a). In contrast, the β-glucan/MUC1 peptide mixture group and the PBS group did not show significant differences, which indicates that the conjugation of β-glucan to the MUC1 peptide is necessary for immune activation. Further analysis of the isotypes and subtypes of the antibodies generated showed that IgG2b is the major subtype (Fig. 4b), indicating the activation of Th1-type response as a ratio of IgG2b/IgG1 > 1.[31] The observed substantial amount of IgM antibodies indicates the involvement of the C3 component of the complement system, which often induces cytotoxicity.[32]

The activity of these anti-MUC1 antibodies was further investigated for their ability to affect tumor cell binding. MCF-7 human breast tumor cells (MUC1 positive) were incubated with antisera from the different vaccinated groups, and the cell surface reactivity was monitored by fluorescence-activated cell sorting (FACS) to measure the amount of cells that bind to the antibodies. The anti-MUC1 antibodies induced by the β-glucan–peptide conjugate showed significantly positive reactivity with MCF-7 (Fig. 4c) in comparison to those induced by the mixture of β-glucan/MUC1 peptide (Fig. 4d). Moreover, the control groups (MUC1 and β-glucan only) did not show significantly different reactivity than the pre-vaccination sera used as the negative control (Fig. S9, ESI†).

To further investigate the immune activation elicited by the synthetic vaccine, we analyzed the expression levels of interleukin-6 (IL-6) and interferon gamma (IFN-γ) in the sera by ELISA. IL-6 is a type-2 (Th2-like) cytokine that plays a role in both innate and adaptive immunity, mediating several aspects of B cell and T cell responses, and promoting antibody production and humoral immunity.[33] IFN-γ is a type-1 (Th1-like) cytokine that has important immunoregulatory properties, including proliferation and differentiation of lymphocyte populations, promotion of NK cell activity and increased antigen presentation. As shown in Fig. 5a and b, the groups that were given the β-glucan component in the vaccine, either mixed or conjugated with MUC1, expressed significantly higher levels of IFN-γ and IL-6 in sera than the PBS group. Furthermore, mice immunized with the β-glucan–MUC1 conjugate exhibited the highest expression levels, with approximately 4-fold increase of IFN-γ and IL-6 in comparison to those immunized only with PBS buffer.

Taken together, the vaccination results demonstrate the ability of the β-glucan–MUC1 conjugate to elicit potent immune responses, as assessed by the production of high IgM and IgG antibody titers reactive against MCF-7. Moreover, the production of both cytokine types, as shown by the high IL-6 and IFN-γ levels observed, suggests the stimulation of Th1 (IFN-γ) as well as Th2 (IL-6) immunity. The immunological results also reveal the poor immunogenicity exhibited by the MUC1 peptide alone, which could be related to the instability of the peptide in circulation that leads to fast degradation and failed antigen delivery. In contrast, the increased antibody titers and immune responses observed in mice immunized with the conjugate could indicate that β-glucan may not only serve as a immunostimulator but also as a carrier facilitating the delivery of the MUC1 antigen within the conjugate to the lymph nodes and other immune organs, where further immune activation will take place. Overall, these results suggest that the β-glucan–MUC1 conjugate could activate both innate and adaptive immunity and could be a stable and effective antigen–carrier construct for vaccine development.

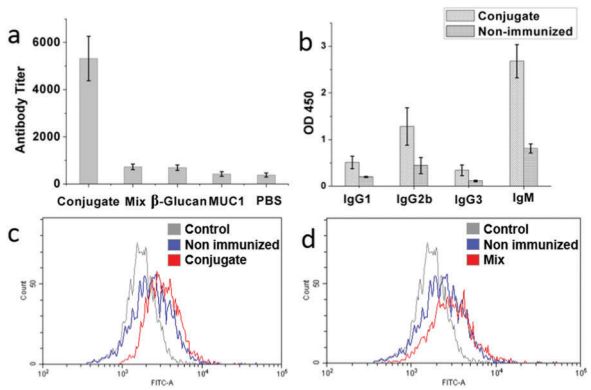


Fig. 4 Immunological evaluation of the synthetic vaccine: (a) antibody titers of different groups. (b) Antibody isotypes and subtypes of the conjugate vaccine. Data are reported as mean ± SD. (c and d) FACS analysis of the binding of antisera induced by the conjugate (c), or the β-glucan/MUC1 peptide mixture (d), to MCF-7 cells, respectively. Grey, blue and red lines represent the cells incubated with PBS buffer, non-immunized sera, and antisera from different experimental groups, respectively.

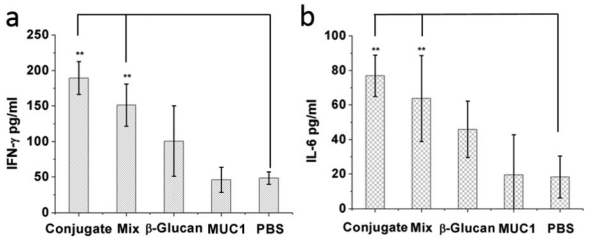


Fig. 5 ELISA test of induced IFN-γ (a) and IL-6 (b) in sera from the vaccinated mice. Data are reported as mean ± SD, $^{**}P < 0.01$.

Published on 03 December 2018. Downloaded by Peking University on 7/7/2020 3:19:44 PM.

In summary, we have developed a novel vaccine construct by covalently linking a synthetic MUC1 peptide to β-glucan, and have studied its structural features as well as immunological properties in mice. The observed diameter and uniform distribution of the conjugate self-assembled nanoparticles can facilitate antigen delivery, recognition and activation of myeloid immune cells, thus resulting in the activation of both innate and adaptive immunity. This is in line with our immunological results, which showed high anti-MUC1 antibody titers cross-reactive with the MCF-7 tumor cell line, as well as high levels of IL-6 and IFN-γ in sera from mice vaccinated with the β-glucan–MUC1 conjugate. In all, our work demonstrates that β-glucan could be a promising carrier and immune activator for increasing the stability and immunogenicity of peptide antigens in the development of novel anticancer vaccine candidates.

The authors are grateful for financial support from the National Key R&D Program of China (2018YFA0507602), the Fundamental Research Funds for the Central Universities: Clinical Medicine + X – Young Scholars Project of Peking University (PKU2018LCXQ003), and the State Key Laboratory of Natural and Biomimetic Drugs. A. F.-T. is a recipient of a Ramón y Cajal fellowship (RYC-2015-17888) and an ERC Starting Grant (ERC-2016-STG-716878). We thank Professor Qin Li, Dr Yuan Wang, Weiqing Zhang, Xulin Sun (Peking University) and Dr Xiumei Wang (Bruker China) for spectroscopic assistance, Professor Yiguang Wang (Peking University) and Dr Xiujing Zheng (Peking University) for helpful discussions.

Conflicts of interest

The authors declare no competing financial interest.

Notes and references

1 R. Rappuoli, M. Pizza, G. Del Giudice and E. De Gregorio, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 12288–12293.
2 I. Melero, G. Gaudernack, W. Gerritsen, C. Huber, G. Parmiani, S. Scholl, N. Thatcher, J. Wagstaff, C. Zielinski, I. Faulkner and H. Mellstedt, *Nat. Rev. Clin. Oncol.*, 2014, **11**, 509–524.
3 S. A. Rosenberg, J. C. Yang and N. P. Restifo, *Nat. Med.*, 2004, **10**, 909–915.
4 G. L. Beatty and W. L. Gladney, *Clin. Cancer Res.*, 2015, **21**, 687–692.
5 Z. Yin, H. G. Nguyen, S. Chowdhury, P. Bentley, M. A. Bruckman, A. Miermont, J. C. Gildersleeve, Q. Wang and X. Huang, *Bioconjugate Chem.*, 2012, **23**, 1694–1703.
6 M. Shi, K. A. Kleski, K. R. Trabbic, J.-P. Bourgault and P. R. Andreana, *J. Am. Chem. Soc.*, 2016, **138**, 14264–14272.
7 H. Cai, Z.-H. Huang, L. Shi, Z.-Y. Sun, Y.-F. Zhao, H. Kunz and Y.-M. Li, *Angew. Chem., Int. Ed.*, 2012, **51**, 1719–1723.
8 R. M. Wilson and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2013, **135**, 14462–14472.
9 M. A. Cheever, J. P. Allison, A. S. Ferris, O. J. Finn, B. M. Hastings, T. T. Hecht, I. Mellman, S. A. Prindiville, J. L. Viner, L. M. Weiner and L. M. Matrisian, *Clin. Cancer Res.*, 2009, **15**, 5323–5337.
10 E. Jäger, D. Jäger and A. Knuth, *Int. J. Cancer*, 2003, **106**, 817–820.
11 S. K. Kim, G. Ragupathi, C. Musselli, S.-J. Choi, Y. S. Park and P. O. Livingston, *Vaccine*, 1999, **18**, 597–603.
12 B. L. Wilkinson, S. Day, L. R. Malins, V. Apostolopoulos and R. J. Payne, *Angew. Chem., Int. Ed.*, 2011, **50**, 1635–1639.
13 A. Kaiser, N. Gaidzik, T. Becker, C. Menge, K. Groh, H. Cai, Y.-M. Li, B. Gerlitzki, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2010, **49**, 3688–3692.
14 S. Ingale, M. A. Wolfert, J. Gaekwad, T. Buskas and G.-J. Boons, *Nat. Chem. Biol.*, 2007, **3**, 663–667.
15 A. L. Parry, N. A. Clemson, J. Ellis, S. S. Bernhard, B. G. Davis and N. R. Cameron, *J. Am. Chem. Soc.*, 2013, **135**, 9362–9365.
16 Z. H. Huang, L. Shi, J. W. Ma, Z. Y. Sun, H. Cai, Y. X. Chen, Y. F. Zhao and Y. M. Li, *J. Am. Chem. Soc.*, 2012, **134**, 8730–8733.
17 Z. Y. Sun, P. G. Chen, Y. F. Liu, B. D. Zhang, J. J. Wu, Y. X. Chen, Y. F. Zhao and Y. M. Li, *Chem. Commun.*, 2016, **52**, 7572–7575.
18 A. Donadei, S. Gallorini, F. Berti, D. T. O'Hagan, R. Adamo and B. C. Baudner, *Mol. Pharmacol.*, 2015, **12**, 1662–1672.
19 J. Tian, J. Ma, K. Ma, H. Guo, S. E. Baidoo, Y. Zhang, J. Yan, L. Lu, H. Xu and S. Wang, *Eur. J. Immunol.*, 2013, **43**, 1220–1230.
20 A. Donadei, S. Gallorini, F. Berti, D. T. O'Hagan, R. Adamo and B. C. Baudner, *Mol. Pharmacol.*, 2015, **12**, 1662–1672.
21 D. Bundle, E. Paszkiewicz, H. Elsaidi, S. Mandal and S. Sarkar, *Molecules*, 2018, **23**, 1961.
22 B. Novakovic, E. Habibi, S. Y. Wang, R. J. Arts, R. Davar, W. Megchelenbrink, B. Kim, T. Kuznetsova, M. Kox, J. Zwaag, F. Matarese, S. J. van Heeringen, E. M. Janssen-Megens, N. Sharifi, C. Wang, F. Keramati, V. Schoonenberg, P. Flicek, L. Clarke, P. Pickkers, S. Heath, I. Gut, M. G. Netea, J. H. Martens, C. Logie and H. G. Stunnenberg, *Cell*, 2016, **167**, 1354–1368.
23 Y. Jin, P. Li and F. Wang, *Vaccine*, 2018, **36**, 5235–5244.
24 G. C.-F. Chan, W. K. Chan and D. M.-Y. Sze, *J. Hematol. Oncol.*, 2009, **2**, 25.
25 G. D. Brown, P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. C. Wong and S. Gordon, *J. Exp. Med.*, 2002, **196**, 407–412.
26 H. S. Goodridge, C. N. Reyes, C. A. Becker, T. R. Katsumoto, J. Ma, A. J. Wolf, N. Bose, A. S. Chan, A. S. Magee, M. E. Danielson, A. Weiss, J. P. Vasilakos and D. M. Underhill, *Nature*, 2011, **472**, 471–475.
27 N. Hassanzadeh-Kiabi, A. Yanez, I. Dang, G. A. Martins, D. M. Underhill and H. S. Goodridge, *J. Immunol.*, 2017, **198**, 375–382.
28 K. Takahara, Y. Yashima, Y. Omatsu, H. Yoshida, Y. Kimura, Y. S. Kang, R. M. Steinman, C. G. Park and K. Inaba, *Int. Immunol.*, 2004, **16**, 819–829.
29 J. Taylor-Papadimitriou, J. Burchell, D. W. Miles and M. Dalziel, *BBA, Mol. Basis Dis.*, 1999, **1455**, 301–313.
30 K. Lin and A. M. Kasko, *ACS Macro Lett.*, 2014, **3**, 652–657.
31 Z. Chen and J. J. O'Shea, *Immunol. Res.*, 2008, **41**, 87–102.
32 R. J. Pleass and J. M. Woof, *Trends Parasitol.*, 2001, **17**, 545–551.
33 T. Fujita, *Nat. Rev. Immunol.*, 2002, **2**, 346–353.

 This journal is © The Royal Society of Chemistry 2019