Broad Spectrum of Neutralization-Enhancement by Novel CD4mimic Compound YIR-821

P-289

Shokichi Takahama[1], Kazuki Tanaka[1], Hasan Zahid[1], Alam Muntasir[1], Takeo Kuwata[1], Ami Masuda[2], Kohei Takahashi[2], Takuya Kobayakawa[2], Takuya Yamamoto[3], Tomoyuki Miura[4], Liya He[5], Hirokazu Tamamura[2], Shilei Ding[6], Andrés Finzi[6] and Shuzo Matsushita[1].

Center for AIDS Research, Kumamoto University, Kumamoto[1], Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo[2], Laboratory of Translational Immunology National Institutes of Biomedical Innovation, Health and Nutrition, Osaka[3], Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto[4], Institute for Molecular Medicine Finland (FIMM), University of Helsinki[5], Centre de Recherche du CHUM, Université de Montréal, Montreal, Quebec.[6]

Abstract

[Background] CD4 mimic compounds (CD4mc) that inhibit the interaction of gp120 with CD4 are expected as an entry inhibitor. Furthermore, CD4mc induce structural changes in gp120 trimer through binding to the CD4 binding (Phe43)-cavity of gp120. We recently developed YIR-821, a novel CD4mc, which showed more potent and less cytotoxic activities than original CD4mc NBD-556. Here we examined anti-HIV-1 activities of YIR-821 including enhancing activities for neutralization and ADCC (antibody-dependent cellular cytotoxicity) against a variety of viruses.

[Methods] We used peudovirus panels of HIV-1 strains (standard panel B/panel C/global standard panel) to investigate the spectrum of YIR-821 for neutralization/binding enhancement as well as activities as an entry inhibitor. Synergistic effects in neutralization were examined for antibodies targeting V3/CD4bs/CD4i regions and calculated by R package “SynergyFinder”. To evaluate ADCC, CEM-NKR-CCR5-luc T-cell line infected with HIV-1 BaL was prepared as target and incubated with antibodies and effector cells (CD16 expressing KHYG-1 NK cell line) for 8 hours, then relative luminescent activity was measured.

[Results] We found broad and potent anti-HIV-1 activities of YIR-821 as an entry inhibitor against panels of subtype-B and C virus. YIR-821 induced exposure of conformational epitopes on gp120 was observed not only in subtypes B and C, but also subtypes A and B/C, which were relatively resistant to the inhibitory activity by YIR-821. Interestingly, YIR-821 treatment alone is not effective against JR-FL (subtype B, but uniquely YIR-821 resistant strain), but the combination of YIR-821 with anti-V3/CD4i antibodies showed synergistically neutralization, suggesting some difference in the process of these two anti-HIV mechanisms. Moreover, we observed potent enhancement for ADCC activity of anti-V3/CD4i antibodies against HIV-1 infected cells in the presence of YIR-821.

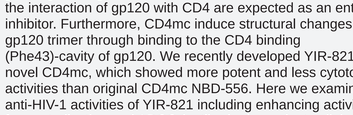
[Conclusions] These data taken together suggest that **YIR-821 has enhancing activity to ADCC with an excellent property of neutralization-enhancing activities for broad spectrum of HIV-1 in vitro.**

Combinational use of YIR-821 and anti-V3/CD4i antibodies represents a promising candidate for prevention or treatment of HIV-1 infection.

Background

Conformation of Env trimmer and CD4mc

Env is a meta-stable complex that transitions from a closed to an open trimer configuration



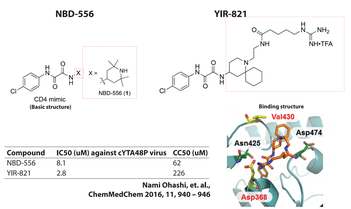
Viral fitness require balance between electropositivity (blue) and hydrophobicity (tan) at the trimer apex CD4 triggers a transition to the CD4-bound conformation with an exposed co-receptor binding site (purple patch)

Schemes modified from Jeong Hyun Lee, et. al., Immunity 46, 690–702, April 18, 2017

Small molecular compound CD4 mimic (CD4mc) was developed to block CD4bs of Env

- # Structurery similar to CD4 molecule
- # Binds to CD4 binding site (CD4bs) of gp120
- # Induce conformational change of gp120
- # Expose CD4-induced epitope (CD4i) or V3

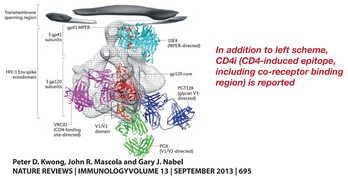
YIR-821 as new CD4mc



Compound	IC50 (uM) against cYTA48P virus	CC50 (uM)
NBD-556	8.1	62
YIR-821	2.8	226

Nami Ohashi, et. al., ChemMedChem 2016, 11, 940 – 946

Epitopes for neutralizing antibodies on Env



In addition to left scheme, CD4i (CD4-induced epitope, including co-receptor binding region) is reported

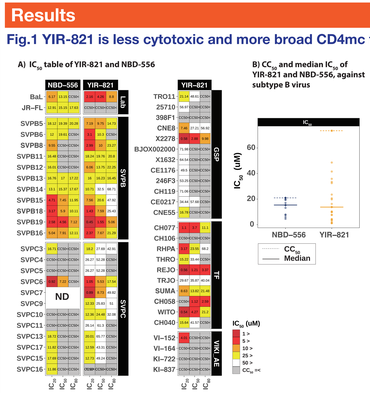
Peter D. Kwong, John R. Mascola and Gary J. Nabel
NATURE REVIEWS | IMMUNOLOGY VOLUME 13 | SEPTEMBER 2013 | 695

In this study, we used

anti-V3 (KD247, 1C10)
anti-CD4i (16B2, 4E9C, 17B)
anti-CD4bs bNAb (VRC01)

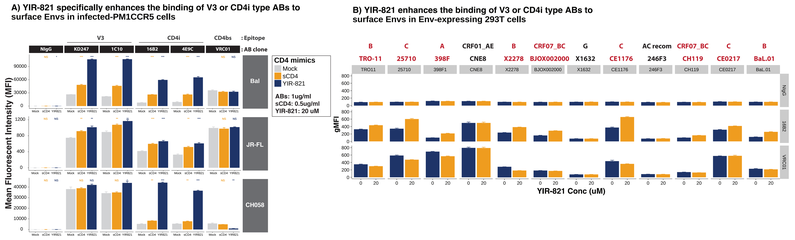
Results

Fig.1 YIR-821 is less cytotoxic and more broad CD4mc than original NBD-556



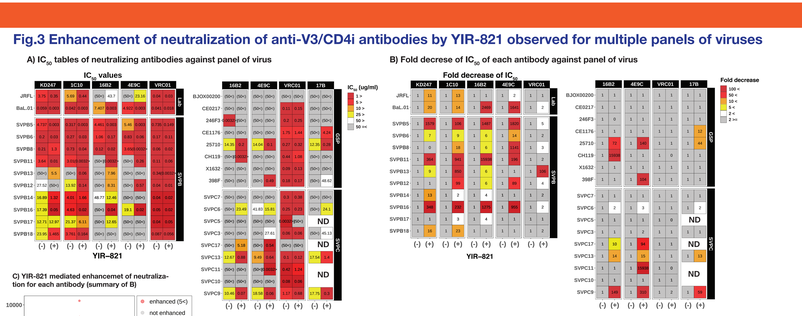
To reveal the spectrum of YIR-821, we examined the YIR-821 activity against multiple panel of HIV-1 strains in TZM-bl-based luc assay.
(A): IC_{50}-IC_{80} and IC_{50} of YIR-821 and NBD-556 against panel of virus were shown.
(B): IC_{50} values of YIR-821 and NBD-556 against panel of subtype-B were plotted. Dashed lines indicate CC_{50} of YIR-821 and NBD-556.
(C): To examine the spectrum of YIR-821, IC_{50} and IC_{50} of YIR-821 against panel of virus were plotted (top) or counted (bottom) with subtypes tested. Red dots indicates the values showing less than CC_{50}.

Fig.2 YIR-821 enhances the bindings of anti-V3 and CD4i, but not CD4bs, antibodies to the cell surface Env



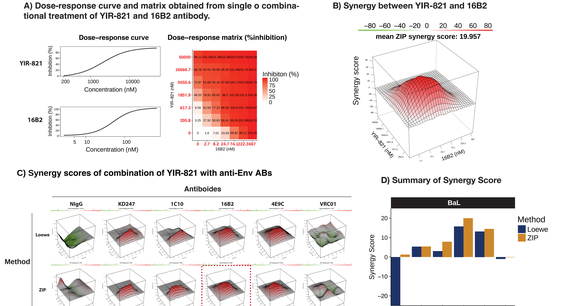
To examine whether YIR-821 induces the conformational change of Env-trimmers from various strains, we examined the binding of anti-Env ABs to Envs on cell surface +/- YIR-821.
In Fig.2-B, red indicate strains sensitive to YIR-821.

Fig.3 Enhancement of neutralization of anti-V3/CD4i antibodies by YIR-821 observed for multiple panels of viruses



To examine YIR-821-mediated neutralization enhancement, we performed neutralization assay of V3/CD4i/CD4bs ABs +/- YIR-821 against multiple panel of HIV-1 strains in TZM-bl-based luc assay.
YIR-821 was used IC_{50} concentrations against each virus strain (determined from the experiment showing Fig.1).
(A): IC_{50} tables of neutralizing antibodies against panel of virus.
(B): To evaluate fold decrease of IC_{50}, fold decrease values of IC_{50} were calculated and plotted (using IC_{50} values without YIR-821 as 1).
(C): To summarize data, values in Fig.3-B were plotted and mean and median values are indicated as solid and dashed lines. More than 5 fold decrease as considered “enhancement” and shown as red dot.

Fig.4 YIR-821 mediated enhancement of neutralization activity of anti-V3/CD4i antibodies occured through synergistic manner



A) To evaluate synergy between YIR-821 and anti-Env antibodies, we first measured the %inhibition in TZM-bl luciferase assay. Fig.4-A shows one of the example of YIR-821 and anti-CD4i antibody(16B2) B) Synergy scores were calculated based on data from Fig.4-A by ZIP method. Red indicates positive synergy, green indicate negative synergy. C) Synergy scores were calculated by two methods independently; (Loewe and ZIP). Red box indicates same image used in Fig.4-B.

Fig.5 Possible mechanism of YIR-821 resistance of JR-FL; involvement of the absense of glycosilation at N197 residue in V2 region

Among 28 subtype B strain tested, only JR-FL showed the resistance to YIR-821. To examine the possible mechanism of that YIR-821 resistance of JR-FL, we first examined the amino acid sequence of subtype-B/C virus strain tested. A) shows the mutation in N197 position (D197N) in JR-FL. This site is known to be glycosylated (Reference below).

B) sensitivity of YIR-821 and soluble CD4 (sCD4) against JR-FL wt or D-197N mutant were examined in TZM-bl luc cells. D197N mutant of JR-FL slightly increased the sensitivity to YIR-821.

C)D197N mutant significantly affect the binding of CD4bs ABs to surface Env, suggesting that N197-glycosylation is involved in the structural property of CD4binding site, which may in turn affecting the YIR-821 sensitivity.

(Reference) N197-glycosylation on Env

Position of N197 and V3 loop in trimmer

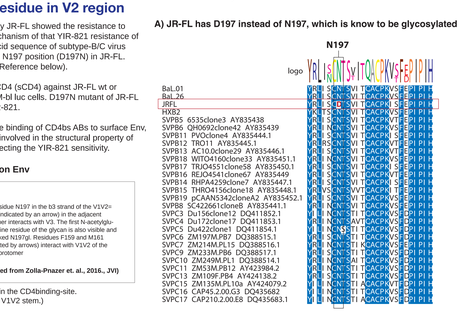


(b) Residue N197 in the b3 strand of the V1V2 stem (indicated by an arrow) in the adjacent protomer interacts with V3. The first N-acetylglucosamine residue of the glycan is also visible and is marked N197gl. Residues F159 and M161 (indicated by arrows) interact with V1V2 of the same protomer

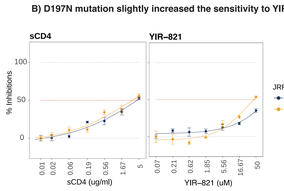
(modified from Zolla-Pnazer et. al., 2016., JVI)

N197 seems not to be directly involved in the CD4binding-site.
(N197 is located in the b3 strand of the V1V2 stem.)

A) JR-FL has D197 instead of N197, which is know to be glycosylated



B) D197N mutation slightly increased the sensitivity to YIR-821



C) D197N mutation significantly decreased the binding to CD4bs-antibody

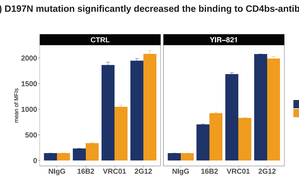
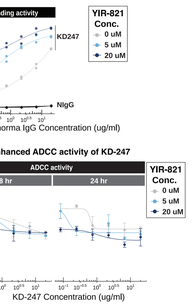
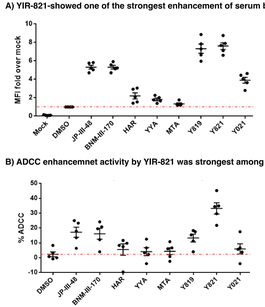


Fig.6 YIR-821 enhanced ADCC activity of KD247 against BaL-infected CEM-NKR-luc cells



To examine YIR-821 mediated enhancement of ADCC,
KD-247 binding to surface Env (A) and ADCC (B) were examine +/- YIR-821.
For ADCC assay, BaL-infected CEM-NKR-CCR5-luc were incubated with hCD16V158 expressing KHYG-1 cells (NK cellline) in the presense or absense of KD247 +/- YIR-821.

Fig.7 Enhancement of ADCC activity against CH58 infected cells by YIR-821



To further examine YIR-821 mediated enhancement of ADCC,
enhancement of ADCC by various CD4mcs were compared by MFI and %ADCC.
A) CH58TF infected HD1514 cells were stained with 5 sera +/- 50 uM CD4mc.
B) CH58TF infected HD1514 cells were incubated with 5 sera +/- 50uM CD4mc, then %ADCC were measured.

Acknowledgement

CEM-NKR-luc cells and hCD16-KHYG-1 cells were kindly provided by Dr. David Evans, This work has been supported by the fund from Japan Agency for Medical Research and Development (AMED)

Contact informations

Shokichi Takahama PhD
(Left, Presenter, shoukichitakahama@gmail.com)
Shuzo Matsushita, MD, PhD
(Right, Corresponding, shuzo@kumamoto-u.ac.jp)

Any comments and suggestions are very wellcome !