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The Optimal Molar Ratio between Binding-Deficient and Fusion-Deficient Murine Leukemia Virus Envelope Proteins for Complementation to Induce Syncytia

Tetsuro Matano*

AIDS Research Center, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashi-murayama, Tokyo 208-0011

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The murine leukemia virus (MLV) envelope protein (Env) plays an important role in inducing membrane fusion during viral entry and cell-to-cell fusion. The ecotropic Friend MLV (FMLV) Env is oligomerized into a trimer in the endoplasmic reticulum, cleaved into the surface protein (SU, gp70) and the transmembrane protein (TM, p15E) in the Golgi apparatus, transported to the cell surface, and incorporated into the virion (1). At the time of virus budding or shortly thereafter, p15E is cleaved into p12E and the C-terminal 16-amino-acid long R-peptide (2). Expression of the wild-type Env does not induce syncytia in NIH 3T3 cells, whereas expression of the R-deleted Env does (3,4). No syncytia are induced by the R-deleted FMLV Env in HeLa cells lacking the FMLV receptor.

Env-mediated membrane fusion is initiated by the binding of SU to the receptor followed by postbinding fusion (1). The receptor-binding domain (RBD) resides in the N-terminal half of SU (5,6). Receptor-binding and postbinding fusion functions can be contributed by two different molecules respectively (7,8). In a previous report (7), a binding-deficient Env with a point mutation in RBD and a fusion-deficient Env with a point mutation in TM functioned in a complementary fasion to induce membrane fusion.

*Corresponding author: Tel: +81-42-561-0771 ext. 335, Fax: +81-42-565-3315, E-mail: matano@nih.go.jp

We previously obtained a chimeric FMLV Env, CD4-Env, the RBD of which was completely replaced with a surface domain of human CD4 (9). The CD4-Env chimera was processed, expressed on the cell surface, and incorporated into the virion. When coexpressed, CD4-Env bound to the wild-type FMLV Env to form hetero-oligomers. Further, CD4-Env contributed the postbinding fusion function and complemented a fusion-deficient TM mutant to induce membrane fusion (10). Because the CD4-Env and the TM mutant are required to form hetero-oligomers in the complementation, the fusion efficiency may be dependent on the molar ratio of the former to the latter. In our previous study, however, we examined the complementation between two molecules only at the molar ratio of 1:1. In this study, the optimal ratio for the complementation to induce syncytia was determined.

pCXN2 plasmids expressing FE (a wild-type ecotropic FMLV Env), FE.T470H (a fusion-deficient FMLV Env with a point mutation in the extracellular domain of TM), R-deleted FE.D86K (a binding-deficient FMLV Env with a point mutation in RBD), and R-deleted CD4-Env, respectively, were used (10,11). Expression of R-deleted FE.D86K did not induce syncytia by itself, but did by co-expression with FE in NIH 3T3 cells. Similar complementation in inducing syncytia was observed in experiments using R-deleted CD4-Env instead of R-deleted FE.D86K. Also, complementation was observed

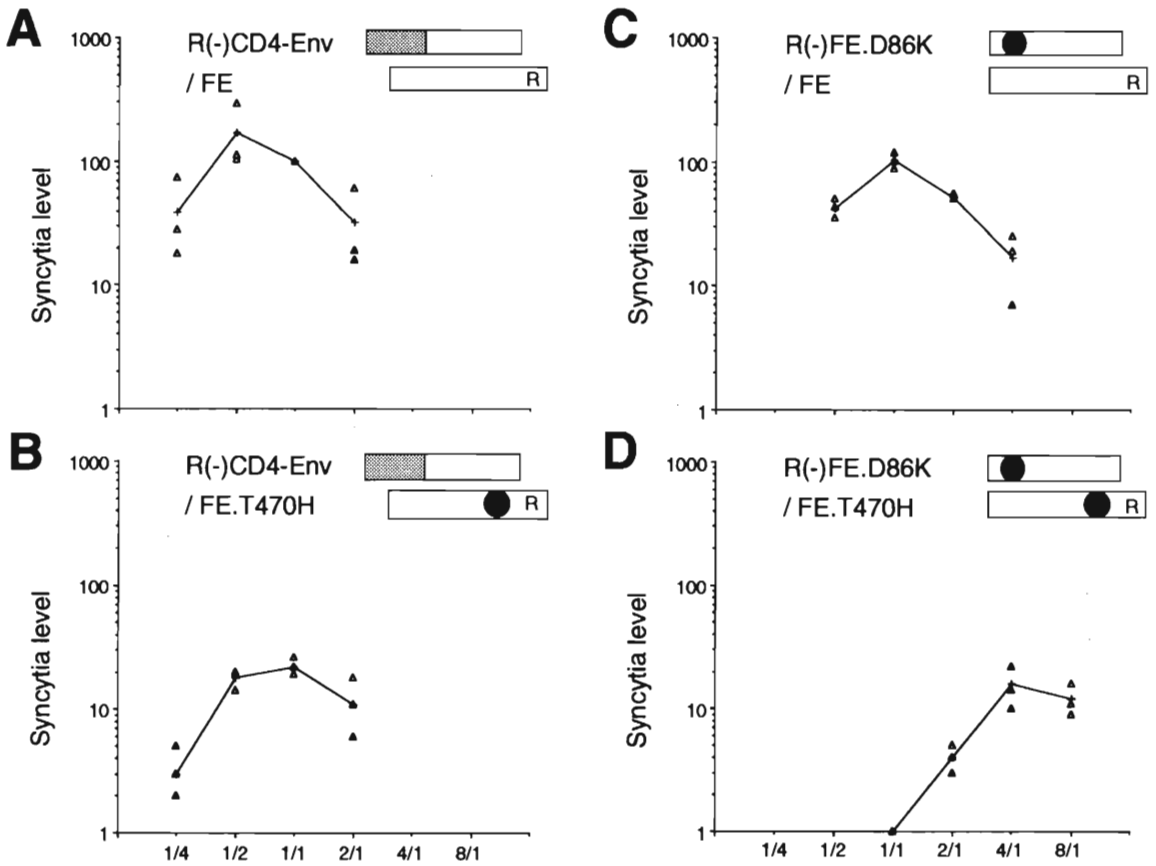

Fig. 1. Syncytia assay on the DNA-transfected HeLa cells cocultured with NIH 3T3 cells. HeLa cells in each well of 6-well plates were cotransfected with different amount ratios of DNAs (2.1 μg in total in each transfection) expressing FE and R-deleted CD4-Env (A), FE.T470H and R-deleted CD4-Env (B), FE and R-deleted FE.D86K (C), or FE.T470H and R-deleted FE.D86K (D). Twenty-four hours after the transfection, the DNA-transfected HeLa cells were diluted serially by two-fold in quadruplicate, and 1×10^5 NIH 3T3 cells were added to 1×10^5 of HeLa cells containing the diluted DNA-transfected HeLa cells per well in 12-well plates. After 24-h coculture, 12-well plates were examined microscopically for syncytia. The number of syncytia produced by the undiluted transfected HeLa cells in each well (in 6-well plates) was calculated as described previously (12). The syncytia level is shown as a value relative to that obtained with the cells transfected with FE and R(-)-CD4-Env-expression plasmids at a 1:1 ratio, which is rated as 100. Each value obtained from each experiment is shown by open triangle. The mean values obtained from three sets of experiments are shown by +.

between FE.T470H and R-deleted FE.D86K or R-deleted CD4-Env. In those complementations, the effect of the molar ratio of the two molecules on induction of syncytia was examined.

First, NIH 3T3 cells were co-transfected with different molar ratios of R-deleted CD4-Env- and FE-expression plasmids, pCXN2R(-)CD4-Env and pCXN2FE, and syncytia induction was compared. Because the nucleotide length of pCXN2R(-)CD4-Env is 8.3 kb and that of pCXN2FE is 8.1 kb, the DNA weight ratio approximates the DNA molar ratio. In NIH 3T3 cells, a pCXN2R(-)CD4-Env-to-pCXN2FE DNA ratio of 1:2 induced syncytia most efficiently (data not shown).

To evaluate the fusion more quantitatively, the syncytia level was examined by co-culture of NIH 3T3 cells with HeLa cells cotransfected with different molar ratios of pCXN2R(-)CD4-Env and pCXN2FE. Co-expression of R-deleted CD4-Env and FE at a ratio of 1:2 induced syncytia most efficiently (Fig. 1A). When R-deleted FE.D86K, instead of R-deleted CD4-Env, was expressed together with FE, the optimal ratio was slightly shifted to the ratio of 1:1 (Fig. 1C), suggesting that R-deleted CD4-Env required more normal RBD *in trans* than did R-deleted FE.D86K for inducing syncytia. When FE.T470H, instead of FE, was expressed together with R-deleted CD4-Env or R-deleted FE.D86K, the syncytia level was greatly reduced and the optimal ratio was shifted to the higher p12E/SU ratio (Fig. 1B and 1D).

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REFERENCES

1. Hunter, E. (1997): Viral entry and receptors. p. 71-120. *In* Coffin, J. M., Hughes, S. H. and Varmus, H. E. (eds), Retroviruses, Cold Spring Harbor Laboratory Press, New York.
2. Green, N., Shinnick, T. M., Witte, O., Ponticelli, A., Sutcliffe, J. G. and Lerner, R. A. (1981): Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polyprotein involves removal of a COOH-terminal peptide. Proc. Natl. Acad. Sci. USA, 78, 6023-6027.
3. Ragheb, J. A. and Anderson, W. F. (1994): pH-independent murine leukemia virus ecotropic envelope-mediated cell fusion: implications for the role of the R peptide and

p12E TM in viral entry. J. Virol., 68, 3220-3231.
4. Rein, A., Mirro, J., Haynes, J. G., Ernst, S. M. and Nagashima, K. (1994): Function of the cytoplasmic domain of a retroviral transmembrane protein: p15E-p2E cleavage activates the membrane fusion capability of the murine leukemia virus Env protein. J. Virol., 68, 1773-1781.
5. Heard, J. M. and Danos, O. (1991): An amino-terminal fragment of the Friend murine leukemia virus envelope glycoprotein binds the ecotropic receptor. J. Virol., 65, 4026-4032.
6. Battini, J. L., Danos, O. and Heard, J. M. (1995): Receptor-binding domain of murine leukemia virus envelope glycoproteins. J. Virol., 69, 713-719.
7. Zhao, Y., Lee, S. and Anderson, W. F. (1997): Functional interactions between monomers of the retroviral envelope protein complex. J. Virol., 71, 6967-6972.
8. Rein, A., Yang, C., Haynes, J. A., Mirro, J. and Compans, R. W. (1998): Evidence for cooperation between murine leukemia virus Env molecules in mixed oligomers. J. Virol., 72, 3432-3435.
9. Matano, T., Odawara, T., Iwamoto, A. and Yoshikura, H. (1995): Targeted infection of a retrovirus bearing a CD4-Env chimera into human cells expressing human immunodeficiency virus type 1. J. Gen. Virol., 76, 3165-3169.
10. Nakamura, H., Takeda, A. and Matano, T. (2001): Postbinding fusion function contributed by a chimeric murine leukemia virus envelope protein. Arch. Virol., (in press).
11. Niwa, H., Yamamura, K. and Miyazaki, J. (1991): Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene, 108, 193-199.
12. Reed, L. J. and Muench, H. (1938): A simple method of estimating fifty per cent endpoints. Am. J. Hyg., 27, 493-497.