Interaction between the Dominant Negative Mutant and the Wild-Type Envelope Proteins of Friend Murine Leukemia Virus

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Interaction between the previously obtained dominant negative mutant, referred to as fcr (T. Matano, T. Odawara, M. Ohshima, H. Yoshinari, and A. Iwamoto, J. Virol. 67:2026-2033, 1993), and the wild-type envelope proteins (Env) of Friend murine leukemia virus was examined. The wild-type Env was bound to the fcr mutant Env and trapped in the endoplasmic reticulum. The virus receptor was not involved in this interaction.

Several types of antiviral strategy using dominant negative mutants, such as the dominant negative rev mutant of human immunodeficiency virus (3, 13, 17, 24), have been studied recently. We previously reported a dominant negative env mutant, referred to as fcr, of Friend murine leukemia virus (FMLV) (19). The fcr mutant env had a point mutation which resulted in a Cys (C)-to-Arg (R) substitution at the 361st amino acid in the FMLV envelope protein (Env). The fcr mutant Env was retained in the endoplasmic reticulum (ER) and accumulated because of its slow degradation. When the fcr gene was transfected into and expressed in cells persistently infected with the ecotropic murine leukemia virus (MLV), the fcr mutant Env trans-dominantly interfered with virus production by inhibiting the intracellular transport of the wild-type Env to the cell surface. The fcr mutant was different from the other previously reported dominant negative env mutants, which had a mutation in the fusion domain or in the receptor-binding domain and were expressed on the cell surface (11, 23). The fcr mutant may lead to the development of an animal model for studying the antiretroviral strategy based on intracellular immunization (4, 12).

Normally, retroviral Env produced in the ER is transported via the Golgi complex and expressed on the cell surface (14, 20, 25). The initial FMLV env gene product in the ER is an Env precursor, SU-TM, detected as pPr90”. The SU-TM, if folded correctly, is multimerized in the ER and transported to the Golgi complex, where it is cleaved into the surface protein (SU, gp70) and the transmembrane protein (TM, p15[E]), which are transported to the cell surface (9, 15). When the wild-type Env was coexpressed with fcr, the processing of the wild-type Env was inhibited at the ER and the processed Env (SU) became undetectable (19).

In this study, we examined the possibility of direct interaction between the wild-type Env and the fcr mutant Env, and to exclude the involvement of the ecotropic MLV receptor in this interaction, the dominant negative effect of the fcr mutant on the processing of wild-type Env in HeLa cells lacking the ecotropic MLV receptor was also examined.

To discriminate the fcr mutant Env from the wild-type Env, the fcr gene (nucleotides 5778 to 7802 according to Friedrich’s numbering [GenBank accession no. X02794] [16]) was tagged in frame with an Lck gene fragment encoding an N-terminal portion (amino acids 5 to 141) of the Lck kinase whose expression is known to be T lymphocyte specific (Fig. 1). The cDNA encoding the wild-type FMLV Env was obtained by reverse transcription and PCR from the RNA extracted from a Friend erythroleukemia cell line, 745a (6, 10, 21). The fcr gene was obtained by reverse transcription and PCR from an immunoselected mutant erythroleukemia cell line, FR-6 (19, 27). The Lck gene fragment was obtained by PCR from a mouse Lck cDNA kindly provided by R. M. Perlmutter (University of Washington) and T. Taniguchi (Osaka University) (18).

The fcr gene tagged with Lck was introduced into the Moloney MLV (MMLV) long terminal repeat-driven env expression plasmid containing a neomycin-resistant marker gene (Fig. 1) (19). This Lck-tagged fcr expression plasmid, pNeFCrL, and the wild-type and fcr mutant FMLV env expression plasmids, pHmFW and pHmFCr, containing a hygromycin B-resistant gene (19) were transfected separately into NIH 3T3 cells by the calcium phosphate method (22) to obtain a G418-resistant cell clone, FCrL1, and hygromycin B-resistant cell clones, FW1 and FCr1, respectively.

Cellular proteins extracted with triplicate-detergent lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.02% sodium azide, 0.1% sodium dodecyl sulfate [SDS], 0.5% sodium deoxycholate, 0.1 mg of phenylmethylsulfonyl fluoride per ml, 1 µg of aprotinin per ml, 1% Nonidet P-40) were analyzed by Western immunoblotting (19, 22). Western blot analysis with the polyclonal goat anti-gp70 (SU) antibody (National Cancer Institute lot 79S00713) recognizing the SU domain of the ecotropic MLV Env showed that FW1 cells expressed the wild-type FMLV Env, which was cleaved into SU, while FCR1 cells expressed the unprocessed mutant SU-TM (Fig. 2A, lanes 1 and 3). In FCR1 cells, the anti-gp70 antibody detected a 110-kDa protein whose size was expected from the unprocessed Env-Lck fusion protein (Fig. 2A, lane 4). The polyclonal

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rabbit anti-human Lck kinase antibody (NT) (Upstate Biotechnology Inc.) which recognizes amino acid residues 22 to 51 of human Lck kinase and has a cross-reactivity with mouse Lck kinase detected the 110-kDa protein only in FCrL1 cells (Fig. 2A, lane 5).

From the lysates of the cells labeled with [35S]methionine for 1 h, the anti-gp70 antibody and the anti-Lck antibody immunoprecipitated proteins with the same molecular mass (110 kDa) (Fig. 2B, lanes 4 and 9). We thus concluded that the 110-kDa protein was the Lck-tagged fcr mutant Env.

To examine whether the fcr proteins bind to each other to form multimers, the fcr expression plasmid (pHmFCr) was transfected into FCrL1 cells and selected with hygromycin B to obtain the cell clone FCrL1/pHmFCr. For immunoprecipitation with the anti-gp70 antibody, cells were lysed with the triple-detergent lysis buffer, while for coimmunoprecipitation with the anti-Lck antibody, cells were lysed with a single-detergent lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.02% sodium azide, 0.1 mg of phenylmethylsulfonyl fluoride per ml, 1 μg of aproamin per ml, 1% Nonidet P-40 (22). The immunoprecipitation analysis using the anti-gp70 antibody showed that FCrL1/pHmFCr1 cells expressed both the nontagged fcr mutant Env and the Lck-tagged fcr mutant Env (Fig. 2B, lane 6). The anti-Lck antibody coimmunoprecipitated the nontagged fcr mutant Env together with the Lck-tagged fcr mutant Env (Fig. 2B, lane 7). This result means that the Lck-tagged fcr mutant Env and the nontagged fcr mutant Env bound to each other, i.e., the fcr mutant Env formed multimers in the ER.

To examine whether the fcr mutant Env binds to the wild-type Env in the ER, the wild-type FMLV env expression plasmid (pHmFW) was transfected into FCrL1 cells and selected with hygromycin B to obtain the cell clone FCrL1/pHmFW1. The immunoprecipitation analysis using the anti-gp70 antibody showed that FCrL1/pHmFW1 cells expressed both the Lck-tagged fcr mutant Env and the unprocessed wild-type SU-TM (Fig. 2B, lane 5). The anti-Lck antibody coimmunoprecipitated the wild-type SU-TM together with the Lck-tagged Env (Fig. 2B, lane 8), indicating that the wild-type Env was bound to the fcr mutant Env and trapped in the ER.

To know whether the ecotropic MLV receptor (1) is involved in the interaction between the wild-type Env and the fcr mutant Env in the ER, we examined the effect of the fcr mutant Env on the processing of the wild-type Env in HeLa cells which express no ecotropic MLV receptor. The wild-type MMLV env expression plasmid (pNeMoW [19]) and the fcr expression plasmid (pHmFCr) were transfected separately into HeLa cells, and a G418-resistant cell clone, HeMoW1, and a hygromycin B-resistant cell clone, HeFCr1, were obtained, respectively. The wild-type MMLV env expression plasmid (pNeMoW) was then transfected into HeFCr1 cells and selected with G418 to obtain the cell clone HeFCr1/pNeMoW1.

The immunoprecipitation analysis using the anti-gp70 antibody showed that HeMoW1 cells expressed the wild-type MMLV Env, which was cleaved into SU, and that HeFCr1 cells expressed only the unprocessed mutant SU-TM (Fig. 2C, lanes 2 and 3). Thus, the wild-type Env was processed normally in HeLa cells while the fcr mutant Env was not. HeFCr1/pNeMoW1 cells expressed both the fcr mutant Env and the wild-type MMLV SU-TM, which migrated slightly more rapidly than FMLV SU-TM in gel electrophoresis (5, 19), but the
wild-type SU-TM in HeFCrI/pNeMoW1 cells was not cleaved into SU (Fig. 2C, lane 4). This result indicates that the processing of the wild-type Env was inhibited trans-dominantly by the fcr mutant Env also in HeLa cells, i.e., the ecotropic MLV receptor was not involved in the dominant negative effect of the fcr mutant on the processing of the wild-type Env. Proper folding of the retroviral Env protein in the ER is essential for its proper multimerization and its transport from the ER to the Golgi complex. Disulfide bonding plays an important role in the folding (2, 7, 8, 26), and its importance in controlling the processing of MLV Env has also been reported (11a). The folding of the fcr mutant Env which had a mutation in a cysteine residue was disrupted, and its transport from the ER to the Golgi complex was inhibited (19). One possible mechanism for this inhibition is that the misfolded fcr mutant Env in the ER fails to form a multimer essential for its transport. Another possibility is that the fcr mutant Env in the ER forms an abnormal multimer which could not be transported into the Golgi complex. In the present study, we confirmed the latter hypothesis, that the fcr mutant Env proteins in the ER bound to each other to form an abnormal multimer.

The dominant negative effect of the fcr mutant was specific to the ecotropic MLV Env. In the ER, where SU-TM is synthesized, and there is an affinity between the virus receptor and SU-TM, it is possible that the dominant negative effect of the fcr mutant on the processing of the wild-type Env is mediated by the virus receptor in the ER. Our observation, however, showed that the fcr mutant did not require the ecotropic MLV receptor for exerting the dominant negative effect on the processing of the wild-type Env.

From these results it is inferred that the wild-type Env binds to the fcr mutant Env in a manner similar to that in which the wild-type Env forms a multimer in the ER but that the multimer consisting of the wild-type Env and the fcr mutant Env is abnormal and unable to be transported to the Golgi complex. Probably, because the protein other than the ecotropic MLV Env would not comultimerize with the ecotropic MLV Env in the ER, the dominant negative effect of the fcr mutant on the processing of the wild-type Env is specific to the ecotropic MLV Env. For the dominant negative effect of the fcr mutant, the ability of the protein to form abnormal multimers with the wild-type ecotropic MLV Env in the ER is essential. Elucidation of the mechanism required for such multimerization may lead to an advance in an antiviral strategy based on intracellular immunization.

For converting our findings into something useful in vivo, delivery of the mutant gene into the target cells and its efficient expression should be worked out. We do not know how much of the mutant protein needs to be expressed to suppress virus infection in vivo. In addition to the way to introduce the fcr gene into the animal, we are currently studying the effect of the fcr mutant on virus infection in the animal model.

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