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Interactions among Measles Virus Hemagglutinin, Fusion Protein and Cell Receptor Signaling Lymphocyte Activation Molecule (SLAM) Indicating a New Fusion-trimer Model

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For measles viruses, fusion on the cell membrane is an important initial step in the entry into the infected cells. The recent research indicated that hemagglutinin firstly leads the conformational changes in the fusion protein then co-mediates the membrane fusion. In the work, we use the co-immunoprecipitation and pull-down techniques to identify the interactions among fusion protein, hemagglutinin and signaling lymphocyte activation molecule (SLAM), which reveal that the three proteins can form a functional complex to mediate the SLAM-dependent fusion. Moreover, under the confocal microscope, fusion protein and hemagglutinin protein can show the cocapping mediated by the SLAM. So fusion protein not only is involved in the fusion but also might directly interact with the SLAM to be a new fusion-trimer model, which might account for the infection mechanism of measles virus.

Keywords: Co-capping, Co-immunoprecipitation, Hemagglutinin of SMD/B, mSLAM, Pull-down, Trimer model

Introduction

Measles virus (MV) is a member of the Morbillivirus genus in the Paramyxoviridae family and has a very limited host range (Bellini *et al.* 1994). Despite vaccination programmes, MV kills approximately 900 000 children each year (Word Health Organization, 2000). MV establishes a systemic infection, which starts from the respiratory tract to a wide range of organs and tissues (Esolen *et al.*, 1993; Takeuchi *et al.*, 2003; Naim *et al.*, 2003). Moreover, MV infection can cause profound immunosuppression, also lead to postinfectious encephalitis, and on rare occasions subacute sclerosing panencephalitis (SSPE), a chronic neurological disorders (Bitnun *et al.*, 1999; Maurice, 2002; Schaulies and Meulen, 2002).

MV entry into target cells is supported by CD46 and SLAM (signaling lymphocyte activation molecule). CD46 ubiquitously expressed on human nuclear cells and was the first cellular receptor identified for MV (Dorig *et al.*, 1993; Naniche *et al.*, 1993). And SLAM expressed on activated lymphocytes, natural killer (NK) cells and mature dendritic cells (Tatsuo *et al.*, 2000; Erlenhoefer *et al.*, 2001; Hsu *et al.*, 2001) was identified the novel MV receptor in 2000. In our previous work, the marmoset SLAM (mSLAM) gene has been screened from the marmoset lymphoid cell cDNA library using the yeast-two-system and identified as the MV receptor in marmoset cells, which had a high homology (86.94% at amino acid level) to human SLAM (Li *et al.*, 2002). However, CD46 is bound only by attenuated vero-cell-adapted wild -type strains, whereas most wild-type MV strains preferentially use the immune-cell-specific protein SLAM as a receptor (Erlenhoefer *et al.*, 2001; Ono *et al.*, 2001; Vongpunsawad *et al.*, 2004). More important, the SLAM-dependent cell entry for MV may be directly related to pathogenesis such as SSPE.

MV contains two surface glycoproteins: the fusion protein (F) and the hemagglutinin (H), which form spikes on the viral envelope and are expressed on the plasma membranes of infected cells (Yao *et al.* 1997). These two proteins are directly involved in virus entry and cytopathology. A number of previous studies (Ebata *et al.*, 1991; Wild *et al.*, 1991; Yao *et al.*, 1997; Plemper *et al.*, 2001) have indicated that both glycoproteins, F and H, participate in the fusion process. H is a type transmembrane glycoprotein that dimerizes in the endoplasmic reticulum. Fusion proteins are synthesized as an inactive precursor, F0, which is activated by a host protease, yielding a transmembrane subunit, F1, and a surface subunit, F2, connected by a disulfide bond (Hsu *et al.*, 1981; Yao *et al.*, 1997; Orit and Yechiel, 2001). The newly formed hydrophobic N terminus of F1 is known as the fusion peptide (Wild and Buckland, 1997; Orit and Yechiel, 200l; Rahaman *et al.*,

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2003). After H binds to the receptor SLAM, it supports fusion of the viral and cellular membranes by inducing a conformational change of the trimeric F protein via the association of both glycoproteins (Baker *et al.,* 1999).

However, there still are some reports that F protein probably can solely mediate fusion and likely act a more important role in the process of the fusion than H protein. Alkhatib *et al*. (Alkhatib *et al.*, 1990) have shown that adenovirus recombinants expressing the MV F gene caused fusion in Human Embryonic Kidney (HEK) 293 Cell cells. In another experiment, some viruses could get the ability of fusion after the F gene of MV was subcloned into these viruses genome (Orit and Yechiel, 2001). These researches indicated that only F protein probably could mediate fusion in some extent. And the analysis of the recent separated MV stains sequence stated that the F protein represent relatively conservative compared with the high variability of H protein (Manie *et al.*, 2000). Meanwhile, the F protein-specific antibodies were more efficient to neutralize MV than that of H protein (Rik *et al.,* 1998). These results reveal that F protein could be more crucial during the MV infecting course.

Thus, the controversy on the roles of F and H is so arresting that it remains to be determined whether F can solely induce the fusion. Although compelling evidence for an interaction between H and F exists (Plemper *et al.,* 2001), it remains unclear on how they interact with the novel receptor SLAM, especially for the interaction between SLAM and F. We have previously reported the interaction between SLAM and H and characterization of the region (aa 429-438) involved in the binding domain of them (Li *et al.,* 2002; Hu *et al.,* 2004). To further explore the interactions between the MV glycoproteins and the receptor SLAM, the co-immunoprecipitation and pull-down assays have been undertaken *in vivo* and *in vitro* respectively. On the other hand, in order to investigate the biological roles of F and H in morphology we transfect either *h* or *f* gene of SMD/B (the separated MV stain derived from the B95a cells) or both respectively in HeLa cells to observe the syncytium formation after they combined with the HeLa cells expressing SLAM. In the previous research work on MV, the smd-h gene derived from the HAD negative MV strain SMD/B is employed in the research, which cannot bind to CD46 because it has an important mutation at position 546 (Li and Qi, 2002).

Materials and Methods

Cells and plasimids CHO (Chinese hamster ovary) cells and HeLa cells, which were kindly presented by professor ZHEN in China Typical Culture Center, Wuhan University, were respectively cultured in F12 and DMEM medium with 10% heat-inactivated newborn calf serum. The plasmids pHM6, pCDNA3.1 and pEGFPN1 are all kindly provided by Dr. Li Lingyun. The plasimd pGEX-6p-1 is kindly provided by Dr. Xiao G.F. And the recombinant plasmid pGADslamΔ779-848 were constructed in our previously work (Li *et al*., 2002).

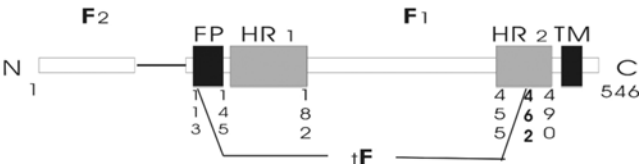

Fig. 1. Schematic representation of the MV F protein and the truncated F (tF) protein. Schematic diagram of the protein showing F1 and F2 subunits connected with a line. Also shown are the positions of the fusion peptide (FP), Heptad Repeat domains (HR1, HR2), tF, and the transmembrane domain (TM) of the F1 subunit.

Recombinant plasmids The *smd/b-h* cDNA gene was cloned in pCDNA3.1,at the double EcorRI site. And primers used to generate pHM6-*f* which can express F protein with HA (Influenza hemagglutin) tag to allow recognition of F protein by a monoclonal antibody specific for the HA tag were P1:5'GAAGCTTGACTCAT CCAATGTCCATCA3' (forward) and P2:5'CGTAGTTCGGGTGG ACCTTAAGAGA3' (reverse) Next, the primers used to generate pGEX-6p-1-slamΔ779-848, pEGFP-slam and pCDNA3.1-SLAM were P3.5'GCGGAATTCATTGGCTGATGGATC3' (forward) and P4.5'ACGCGTCGACTCTCTGGTGTCAGCTC3' (reverse). At the same time, the primers P5.5'AAACTCGAGATGTTTGCGGGAG TAGTCCTG3' (forward) and P6.5'CCCAAGCTTTTAGCATCCT CCAACTTAGCA3' (reverse) were used to amplify truncated f (tf) gene and cloned in to the pHM6, in which the tf is a sequence deleting the intracellular and transmembrane domain of F protein (see Fig 1). All the recombinant plasmids have been confirmed by DNA sequencing.

Antibodies The monoclonal antibody specific for SLAM and HA tag were purchased from the Santa Cruz Company and the Roche Company respectively. And we injected the purified H protein expressed in the *E. coli*. into the rabbit to get the antiserum against H protein. Texas red labelled goat anti-rabbit antibody and FITC (Fluoreszeinthiocyanat)-conjugated goat anti-mouse antibody and AMCA (Aminomethylcoumarin acetic acid) conjugated donkey anti-SLAM were all purchased from the SanYing Company, Wuhan city, China.

Expression and purification of SLAMΔ779-848 protein The construction of the pGEX-6p-1-slamΔ779-848 was confirmed by DNA sequencing. Proteins were expressed in *Escherichia coli* and affinity-purified on glutathione-Sepharose-4B beads (Amersham Biosciences, Richmond, USA) according to the manufacturer's protocols. Bound proteins were eluted using 30 mM glutathione in 50 mM Tris and 100 mM Nacl, pH8.0. Proteins were concentrated and removed salinity prior to use in binding studies.

Transfection and pull-down experiment CHO cells were cultured in the T-75 cell culture bottle under the condition described above. For transient protein expression, cells were grown to 70-80% confluency and co-transfected with 10 μg of the pCDNA3.1-h and pHM6-*f* by means of LipofectAMINE (20 μl) (Invitrogen, San Diego, USA) according to the manufacturer's protocols. Cells were generally washed in PBS and lysed for 30 min at 4°C in lysis buffer (Pierce, Rockford, USA) after 48-72 h transfection.

For pull-down experiment, the CHO cells lysate containing the total proteins were mixed with 50 μg GST (glutathione S Transferase)-fused SLAMΔ779-848 generated previously. Protein complexes were pelleted using 50 μl glutathione-Sepharose-4B beads, washed five times with PBS buffer, resuspended in 1 × SDS loading buffer, and resolved on 12% SDS-polyacrylamide gels. Protein bands were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, USA) and incubated in a blocking solution of 5% skin milk for 1 h at room temperature. The membrane was then washed three times in TBS and probed with monoclonal anti-HA and rabbit anti-H serum respectively for 1 h at room temperature. After 3 times washing with TBS, the membrane was incubated in the AP-conjugated secondary antibody and analysed by enhanced chemiluminescence (Pierce).

Co-immunoprecipitation and Western Blot The CHO cells co-transfected with the three groups, pHM6-*f* and pCDNA3.1-h (group 1); pHM6-*f* and pEGFP-slam (group 2); pHM6-tf and pEGFP-slam (group 3), then lysed with the Mammalian Protein Extraction Reagent (Pierce co-immunoprecipitation kit). According to the protocol of the co-IP Kit, the lysate incubated with the Immobilized Protein G for 2 h at the 37°C. F protein was precipitated with monoclonal antibodies specific to HA tag. The precipitation proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Milipore). And membranes were probed sequentially with antiserum specific to H or the monoclonal antibody specific to SLAM, then analysed by enhanced chemiluminescence (Pierce).

Cell surface immunofluorescence and fusion assay HeLa cells were grown on glass coverslips in 6-well plates without antibiotics and allowed to reach ~80% confluence prior to transfection. Equal amounts (5 μg) of either pCDNA3.1-h or pHM6-*f* or both, respectively, were transfected into the cells by using LipofectAMINE2000 (Invitrogen) according to the manufacturer's protocol. Meanwhile, the pCDNA3.1-SLAM was also transfected into the cells in other wells. Sixteen hours posttransfection, cells were washed with ice-cold PBS three times before being added rabbit anti-H or mouse anti-HA or both accordingly. Besides, the HeLa-SLAM cells were added goat anti-SLAM. And cells were incubated for 2hrs.After PBS washing, Texas red labeled goat anti-rabbit antibody or FITC-conjugated goat anti-mouse antibody or AMCA conjugated donkey anti-goat was added respectively and cells were incubated for another 40 min. Then the HeLa-SLAM cells mixed with the other three group cells and incubated at 37°C for 30 min. After three times PBS washing, the cells continued to be cultured for 24 h. Then the fusion effect on the whole surface of the cells was observed.

Cocapping assay In generally, confluent monolayers of cells were cotransfected with pHM6-f, pCDNA3.1-h and pCDNA3.1-SLAM.24 h posttransfection, cells were washed with PBS three times before being fixed with 2% paraformaldehyde in PBS. The rabbit anti-H and mouse anti-HA added onto cell monolayers, and the cells were then incubated at 37°C for 2 h. After three times PBS washing, the cells added Texas red-labeled goat anti-rabbit antibody and FITC-conjugated goat anti-mouse antibody incubated at 37°C for 40 min. The cells were then examined with a confocal microscope.

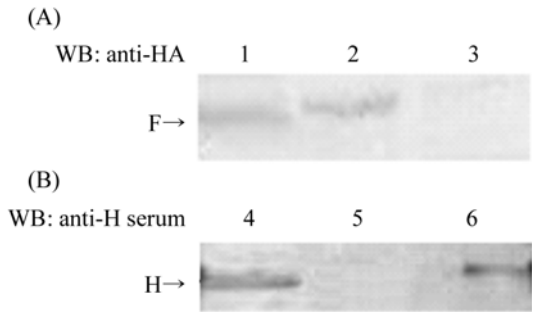

Fig. 2. Binding of SLAM,H and F assayed in a pull-down study using GST- SLAMΔ779-848, H and F. The CHO cells were co-transfected with 10 ug of the pCDNA3.1-h and pHM6-f using LipofectAMINE2000. After 48-72 hrs transfection, the cells lysates containing the total proteins were pull-down by the GST-fused SLAMΔ779-848. The Glutathione Sepharose beads combined with the cell lysates (lane 2 and 6) and were detected by the anti-HA and anti-H serum respectively, which were represented as negative controls. The cell lysates were pulled-down by the GST-fused SLAMΔ779-848 and were detected by the anti-HA (lane 1) and the anti-H serum (lane 4) respectively. The direct cell lysates were detected with the anti-HA (lane 2) and anti-H serum (lane 6) as the positive control.

Results

Formation of the complex of SLAM, H and F *in vitro* If the H protein is necessary for the SLAM-induced fusion process, the H protein should either indirectly or directly interact with SLAM and F glycoprotein. To explore the latter possibility, we employed a purified SLAMΔ779-848 protein with GST-fusion in an attempt to pull down the H and F from the lysate of CHO cells that transiently express H and F plasmids. As seen in Fig. 2-A-1, the F can be detected using the monoclonal antibody specific to the HA tag labeling with the F protein. Similarly in Fig. 2-B, the H protein can also be detected by the rabbit anti-H serum (Fig. 2-B 4). Meanwhile, the direct cell lysates containing the H and F represent as the positive control respectively (Fig. 2-A lane 2 and Fig. 2-B lane 6). Moreover, the GST-sepharose4B beads (Fig. 2-A lane 3 and Fig. 2-B lane 5) cannot pull down either H or F protein in the absence of the GST-SLAMΔ779-848 indicating that SLAM interacts specifically with H and F in vitro and these proteins could form a trimer complex.

But the interaction among these proteins in the complex still has several possibilities. Perhaps, the F protein have been pulled-down by the GST-SLAMΔ779-848 just via the association with the H protein, whereas it cannot directly interact with the SLAM by itself. To further investigate the status of the every protein in detail, the co-immunoprecipitation of H and F, F and SLAM respectively *in vivo* have been pursued.

H protein can interact with F protein *in vivo* To investigate the interactions of the F and H proteins, the co-immunoprecipitation assay of H and F was employed to

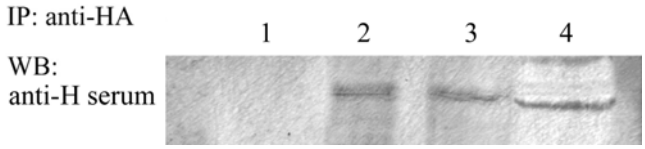

Fig. 3. Co-immunoprecipitate (*IP*) assay identify the interaction between H and F proteins. The CHO cells co-transfected with the pHM6-f and pCDNA3.1-h were lysed with the Mammalian Protein Extraction Reagent and incubated with the Immobilized Protein G for 2 hrs at the 37°C. The lysates immunoprecipited by the anti-HA (lane 2 and 3) were detected by the anti-H serum. The cell lysates combining with the Immobilized Protein G in the absence of anti-HA were detected with anti-H serum indicated the negative control, while the cell lysates were directly detected with anti-H serum indicated positive control.

identify this physical association. In Fig. 3, the CHO cells were cotrasfected with the pHM6-*f* and pCDNA3.1-h. According to the protocol of the co-IP Kit, the cell lysates containing the surface proteins were immunoprecipted with the monoclonal anti-HA (specific to the F protein). The result demonstrated that the H protein (Fig. 3 lane 2 and 3) could be clear detected using the rabbit anti-H serum, in which the proteins added in lane 2 and 3, are all the same outcomes from co-IP assay, whereas the simple protein G without anti-HA could immunopricipitate nothing (Fig. 3 lane 1). Meanwhile, the direct cell lysates were blotted by anti-H as the positive control (Fig. 3 lane 4). The result indicated that the H and F protein could physically associate to form a functional complex that is critical for syncytium formation *in vivo*, which is also consistent to the reports of some researches (Plemper *et al.*, 2001; Vongpunsawad *et al.,* 2004). It seems that the association of H and F can afford an excellent basis for the potential conformal change of the F protein.

The interaction of both SLAM and F glycoprotein *in vivo*, but the tF are deficient in binding to SLAM To find out whether SLAM and F can interact each other *in vivo*, we also have done the co-immunoprecipitation experiment using the monoclonal anti-HA specific to the F protein. The result is revealed in the Fig. 4A. The SLAM protein can be detected using the polyclonal anti-SLAM (lane 2), but the lane 1 show nothing in which the cell lysates were just mixed with the protein G without adding anti-HA in the process of co-IP experiment. It demonstrates that the F protein can interact specifically with the SLAM *in vivo*.

On the same time, the pEGFP-SLAM and pHM6-tf that only encodes the part of ectomembrane of the whole F protein (see Fig. 1) cotransfected into the CHO cells and the co-immunoprecipitation of them have been pursued in order to primarily identify the binding domain of the F protein interacting with the SLAM. The result of co-immunoprecitation of the tF and SLAM is showed in Fig. 4B. Nothing can be detected using the anti-SLAM except the positive control

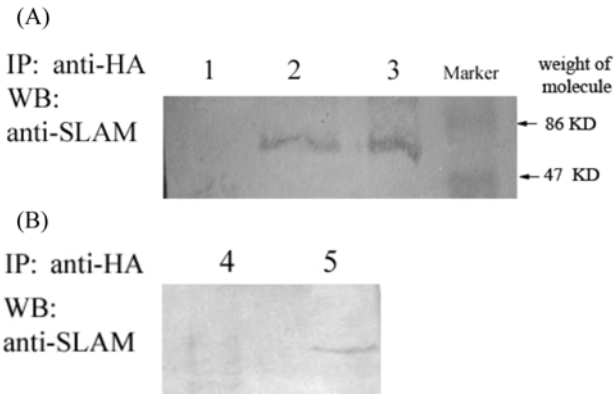

Fig. 4. The co-immunoprecipitation of F and SLAM (A), tF and SLAM (B). The CHO cells co-transfected with the two groups, pHM6-f and pEGFP-slam (Fig. 4A); pHM6-tf and pEGFP-slam (Fig. 4B), were lysed with the Mammalian Protein Extraction Reagent and incubated with the Immobilized Protein G for 2 hrs at the 37°C. The cell lysates containing the SLAM and F or tF immunoprecipitated by anti-HA, then the resulting proteins were blotted with the anti-SLAM (lane 2 and 5). The Immobilized Protein G combining with the cell lysates in the absent of anti-HA (lane 1) and the direct cell lysates (lane 3 and lane 5) were as the negative and positive control respectively. The SLAM can be precipitated by F (lane 2) but not by tF (lane 4), and the Prestaining Protein Marker was used to indicate the range of molecular weight of the band (Fig. 4A).

(Fig. 4 lane-5), which states that the tF cannot interact the SLAM. Because the tF protein includes the complete HR1 domain and only a part of HR2 domain, the result also indicated that the HR2 (aa 438-488 of F see Fig. 1) domain was probably to be essential to the interaction of F and SLAM which was consistent with some other reports that the HR2 domain would be more important to the Cell Fusion than HR1 domain (Lambert *et al.,* 1996; Rahaman *et al.,* 2003).

Co-expression of F and H can induce the fusion To further study the roles of the tee proteins in the process of fusion, some work on the cell morphology has been done to observe the exact role of either F or H protein to the cell fusion. The result indicated that the HeLa-SLAM cells combining with the HeLa-H cells show the cells adsorption rather than the syncytium formation. As shown from Fig. 5A-1, some cells expressing the H congregate onto the monolayer HeLa-SLAM cells observed in the light sight; the Texas red-labelling H (Fig. 5A-2) and AMCA-labelling SLAM (Fig. 5A-3) further ensure the result observed under the fluorescent microscope. In the contrast, the F protein alone seems to be little effect to the cell fusion, neither adsorption nor fusion occurs (Fig. 5B). On the contrary, the HeLa-SLAM cells combining with the cells co-expressing the F and H show the formation of syncytium (Fig. 5C). Of these figures, the Fig. 5C-2 and Fig. 5C-3 are observed under the fluorescence microscope.

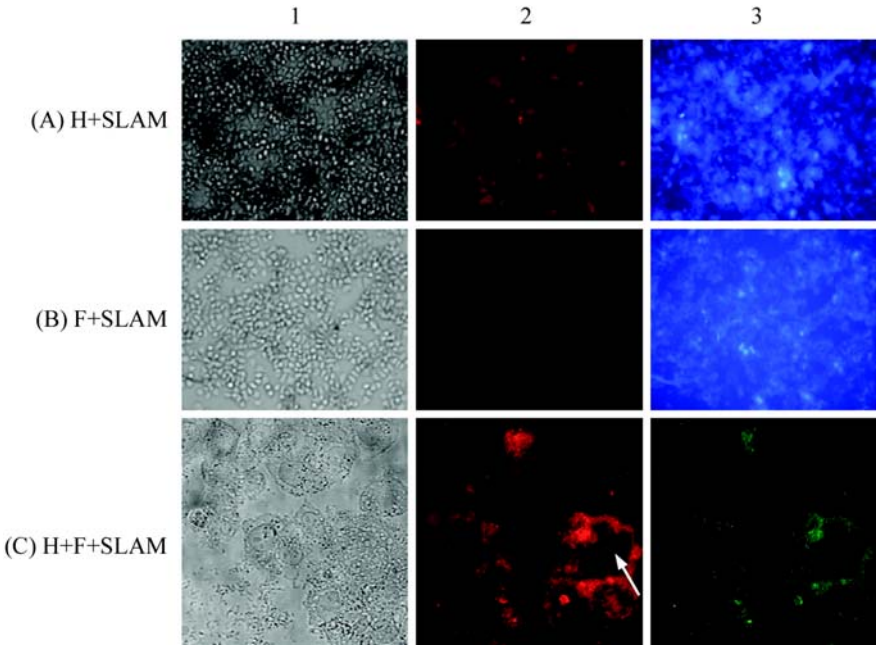

Fig. 5. Effect of H, F and F/H cotransfected HeLa cells on cell fusion. Lane 1 is observed in the light sight and the lane 2 and lane 3 are observed under the fluorescence microscope. The H alone shows the adsorption when it mixed with SLAM (A): the red cells represented the cells expressing the H protein (A-2) which are adsorbed by the blue cells expressing the SLAM (A-3). But the F alone shows little effect mixing with the SLAM (B-1, 2, 3). Only co-expressing the H and F can induce the fusion (marked by white arrow) when mixing with the SLAM-HeLa cells (C): the H protein (C-2) was marked with the Texas Red and the F protein (C-3) with the FITC.

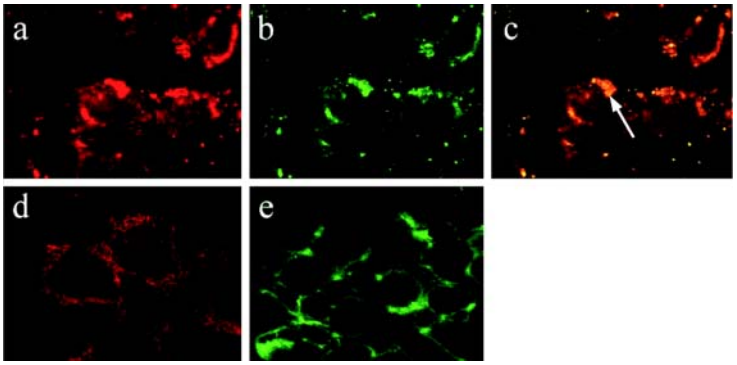

Fig. 6. Cocapping of F and H proteins. HeLa cells were cotransfected with F, H and SLAM (a, b, c) or H alone (d) or F alone (e). At 24 h posttransfection, the HeLa cells were first incubated with rabbit anti-H antiserum and anti-HA at 37°C for 2 hrs and then with Texas red-labeled goat anti-rabbit antibody and FITC-conjugated goat anti-mouse antibody incubated at 37°C for 40 min. Cells were examined with a confocal microscope. F and H cotransfected cells showed Texas red staining of a red cap formed by H (a), FITC staining showing cocapping of F (b), and an image corresponding to F and H in the same focal plane is shown in panel c (marked by white arrow). The yellow cap shows that both the F and H proteins cocap in the same position. But the H or F alone (d or e) showed to uniformly distribute on the cell surface.

Cocapping of H and F glycoproteins of MV Many viral glycoproteins have the potential to undergo receptor induced lateral redistribution on plasma membranes to form a discrete cap on the cell surface (Yao *et al.*, 1997). To further study the interactions of the F and H proteins on the cell surface, capping and cocapping assays were performed. In common conditions, the F or H alone will remain evenly distributed around the cell surface (no capping). In contrast, if under conditions coexpressing the H and F on the cell surface, both H and F can form the capping. Moreover, the F proteins will migrate to the same position (cocapping) on the cell surface as H proteins. This would indicate that these two proteins are physically associated with each other. Figure 6a shows the red cap formed by H, while Fig. 6b shows a green cap formed by F. On the surface of the doubly stained cells (Fig. 6c), it can be seen that the two membrane proteins are co-redistributed to

form the polar caps in the same position. In contrast, as shown in Fig. 6d and Fig 6e, the Texas red-stained H or FITC-stained F remained uniformly distributed on the cell surface.

Discussion

Apparently, fusion as one of the initial steps of viral infection is an obvious target for antiviral agents; the actual mechanism in terms of the proteins involved and their conformational changes have been a hot topic in recent research. The controversy on whether F can solely induce the fusion sounds like an arresting issue. In the morphologic work, the H alone seems to show adsorption rather than fusion, whereas F alone even has little effect on both adsorption and fusion. Only coexpressing H and F, the fusion occurs.

To further understand this issue, the research on the interactions of MV glycoproteins and its receptor SLAM shows more important. The pull-down assay *in vitro* on this three proteins demonstrated they could form a trimer that was perhaps essential to the fusion process. And the co-immunoprecipitation assay between the glycoproteins and SLAM each other has been performed *in vivo* to reveal the detailed mechanism of MV infection. The interaction of the H and F proteins has been identified in present study, which states the physical association of these two proteins. And several mechanisms have been raised including that the association could result in the conformational change of F protein, which leads the fusion peptide involve the C-terminal half of the HR-2 helix exposure (Helene *et al.,* 1999). In other words, the H protein is seemed necessary for the fusion of MV.

Besides, the interaction between F and SLAM has also been identified, which suppose that the mechanism of fusion of MV seems more complex than simple conformal change induced by association of F and H. We still believe that the physical association of the F and SLAM is also a crucial step during the process of fusion, which perhaps also results in the second conformal change of F protein after binding of H and SLAM. Only when the trimer model of F, H and SLAM is formed, the fusion can efficiently accomplish. Shown from the co-capping assay, the H or F alone seems not to form the cap unless both of them combine with SLAM. Moreover, the caps formed by them would be migrated in the same place. Although F can induce the fusion at last, the F alone absent of H protein usually evenly distributed onto the cell surface so that it cannot efficiently exert its fusion function. It states that H protein is more likely to promote the fusion efficient but not determine this process. And the F protein specific antibodies are more efficient to neutralize MV than that of H protein (Rik *et al.,* 1998), which can also consistent with our suspect.

Indeed, there are some reports that single F protein can still mediate the fusion via recombinant virus (Alkhatib *et al.,* 1990; Orit and Yechiel, 2001). It sounded reasonable to some extent because of the direct interaction between F and SLAM. But we believe this special situation is based on the high intensity of F protein in the case of recombinant virus to facilitate its contact with the receptor SLAM. In the common condition, the H protein still acts an important role in the whole fusion process. It is believed that the H protein can greatly promote the efficiency of fusion. It is possible that the association of H and F protein resulting in the conformal change of F is helpful for the binding with SLAM. So the cells expressing the F alone have little effect of fusion in general.

There have been some researches on the identification of characteristic regions of fusion protein because of its special role in the process of MV infection. Robin Buckland and his coworkers (Buckland *et al.,* 1992) primarily found the leucine zipper structure in the F protein is essential for fusion. Later, they reported that a peptide corresponding to the leucine zipper region (amino acids 455-490) could inhibit the MV entry the cell but not affect the attachment of MV, whereas a peptide to amino acids 148-177, corresponding to the amphipathic a-helix region, could not (Wild and Buckland., 1997). Furthermore, the two heptad repeat domains including aa 116-191 (HR1 see Fig. 1) and aa 438-488 (HR2) were identified and the HR2 peptide was the potent inhibitor of MV fusion (Lambert *et al.,* 1996). Similar result in the research on the F protein of PPRV (Peste des petits ruminants virus) was reported that both HR1 and HR2 inhibit PPRV-mediated syncytia formation in Vero cells *in vitro*. Of these, HR2 was found to be more effective than HR1 (Rahaman *et al.,* 2003). Furthermore, T. Fabian reported that F455-470 failed to inhibit MV-induced fusion in Vero cells at concentrations up to 400 μM, whereas F468-487 inhibited fusion at 400 μM and partially at 200 μM. When the two peptides were mixed together to test for synergic effects, fusion was not inhibited at concentrations up to 100 μM (Wild and Buckland, 1997). This result was consistent with that the whole F protein can interact with the MV receptor SLAM while the tF (aa 113-462) cannot. It is possible that the HR2 domain is essential for the interaction between F and SLAM. Moreover, it is likely proposed that the peptide from the HR2 prevents fusion by competing with the F protein for the receptor SLAM. Of course, the exact region of the interaction of F and SLAM is still obscure and this part of work is deserved to pursue.

In conclusion, the association of H and F could result in the first conformational change of F protein, which leads the fusion peptide involve the C-terminal half of the HR-2 helix exposure (Helene *et al.,* 1999). Then the F can interact with the SLAM perhaps inducing the second conformational change among them, which results in the stable trimer formation. It is significant to understand the mechanism of MV infection not only in the basic theory research but also in the applicant in clinic therapy. In the case of the better conservation of F than that of H, the medicine designed according to F protein seems more attractive. And recently some small-molecular MV inhibitor based on the F protein has been reported (Plemper *et al.,* 2004). In addition, the

functional model can also give an illumination to the analysis of the mechanism of paramyxovirus entry and might contribute to our understanding of F protein-mediated membrane fusion because the overall structures of the F proteins are conserved in the paramyxovirus family.

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