Cell-to-cell contact via measles virus haemagglutinin–CD46 interaction triggers CD46 downregulation

Slavica Krantic,* Cyrille Gimenez and Chantal Rabourdin-Combe

Laboratoire de Biologie Moléculaire et Cellulaire, UMR49, Ecole Normale Supérieure, 46 Allée d’Italie, 69634 Lyon, Cédex 07, France

CD46 downregulation by measles virus (MV) occurs after expression of virus haemagglutinin (H) protein on the surface of the infected cell and is a consequence of CD46–H interaction on the cell membrane. To assess whether CD46 downregulation also occurs after CD46–H interaction when these two molecules are expressed on distinct cells, we used human T cell line Jurkat (expressing CD46) and transfected murine fibroblast line L stably expressing MV-H protein (L.H). FACS analysis shows that cell-to-cell contact of 1 h at 37 °C triggers a reduction of CD46 cell surface labelling as detected by MCI20.6, GB24 and J4-48 monoclonal antibodies. This reduction is similar to that observed after MV infection or after infection with recombinant vaccinia virus encoding MV-H protein. By contrast, MV-H protein was downregulated only when CD46–H interaction occurred on the same cell membrane. CD46 downregulation is specific for CD46–H interaction because it was not observed after co-incubation of Jurkat cells with either L cells expressing MV nucleoprotein (L.NP) or L cells. Moreover, this downregulation could be blocked by either anti-CD46 or anti-H antibodies. The H-mediated CD46 downregulation is reversible and restricted to CD46 since expression of other surface markers (CD3, CD14, CD47 and CD63) is unaffected. It is apparently not mediated in a protein kinase (PK) A- or PKC-dependent manner. Altogether, our results provide an unequivocal demonstration that interaction between the extracellular domains of CD46 and MV-H is sufficient to trigger CD46 downregulation.

Introduction

Measles is an acute respiratory tract infection and is still one of the primary causes of infant mortality in developing countries (Bloom, 1989). Lethal complications of measles virus (MV) infections are a consequence of transient but severe MV-induced immunodepression (Norrby & Oxman, 1990). One of the best documented aspects of MV-dependent immunodepression is impaired ability of infected lymphocytes to respond to mitogen activation (McChesney et al., 1987, 1988; Yanagi et al., 1992). The cellular and molecular mechanisms responsible for MV-related immunodepression are as yet poorly understood.

CD46 (or membrane cofactor protein) has recently been identified as a MV cell receptor; its expression is restricted to human and some simian cells (Naniche et al., 1993a; Dörig et al., 1993; Maisner et al., 1994; Manchester et al., 1994). All isoforms of CD46 share four N-terminal short consensus repeats (SCR), a juxtaposed plasma membrane region and a short transmembrane region. CD46 isoforms differ in their STP (Ser, Thr, Pro) -rich regions (STP-A, B, C) and in their cytoplasmic tails (CYT1 and CYT2) (Russel et al., 1992). Under physiological conditions, CD46 serves as receptor for cell membrane-bound C3b/C4b. It acts as a cofactor of the plasma serine protease factor I to cleave C3b and C4b and therefore protects cells from autologous complement-mediated lysis (Liszewski et al., 1991).

During MV infection, viral envelope glycoprotein haemagglutinin (H) is the ligand of CD46 (Gerlier et al., 1994a; Maisner et al., 1994). It has also been reported that, during MV infection, concomitantly with the appearance of MV-H protein on the cell surface, CD46 is downregulated by internalization (Naniche et al., 1993b). Moreover, all CD46 isoforms (B-CYT2, C-CYT2, BC-CYT1 and BC-CYT-2) tested are downregulated from the cell surface after MV infection (Gerlier et al., 1994b). Such downregulation is also observed when H is expressed after infection with a recombinant vaccinia virus encoding the MV-H protein (Naniche et al., 1993b). In this latter case, it appears clear
Fig. 1. For legend see opposite.
that CD46-H interaction triggers CD46 downregulation when both molecules are expressed on the surface of the same cell. However, it remained unknown whether CD46 downregulation during MV infection could occur also between CD46 and MV-H expressed on the surface of uninfected and infected cells, respectively. Indeed, during MV infection, both types of interaction can occur. To address this possibility, we studied CD46 downregulation by using two different cell types expressing either CD46 (human Jurkat T cell line) or MV-H protein (murine L fibroblast cell line stably expressing MV-H protein). Our data show that cell-to-cell contact triggers CD46 downregulation provided that CD46–H protein interaction takes place.

**Methods**

**Transfections.** L.H and L.NP cell lines were obtained by transfection of parental LTK− cells with expression vectors encoding, respectively, Hallé strain MV-H and MV-NP proteins as previously described (Lombard-Plalet et al., 1993).

**Coculture procedure.** All cell lines used in the experiments were grown in Dulbecco's modified Eagle's medium supplemented with 6% fetal calf serum, 10 mM-HEPES, 2 mM-glutamine, 50 μM-2-mercaptoethanol and 50 μg/ml gentamicin.

To set up this experimental model system, we took advantage of different properties of fibroblastic (adherent) and lymphoma (non-adherent) cells, since after coculture for a given period of time, the two cell types can be easily separated. Jurkat cells and L.H (or either L.NP or L, in control experiments) cells were cocultured at 1:1 cell ratio in plastic flasks (Costar) at a total density of 1 × 10⁶ cell/ml. After different time periods, Jurkat cells were collected by simple pipette aspiration after vigorous hand tapping of the flask. The flask bottom was rinsed twice with culture medium and L.H cells were then detached by EDTA treatment (1 mM-EDTA for 2 min at 37 °C). Both cell types were further washed in PBS containing 1% BSA and 0.1% Na₃citrate and analysed by flow cytometry (for details, see Naniche et al., 1992a).

To assure that Jurkat and L.H cells were properly separated after the coculture, we systematically checked the efficiency of separation by searching for the presence of CD46 on L.H cells. Conversely, the presence of MV-H protein on the surface of Jurkat cells was also studied. A cross-contamination of one cell type by the other was never detected (data not shown).

However, the adherent properties of the cells used are of fundamental importance in this model system. For example, when semi-adherent mastocytoma cells stably expressing MV-H protein (P815.H) were cocultured with fibroblastic cells (HeLa), the subsequent separation of the two cell types was not satisfactory. Indeed, HeLa cells cocultured with P815.H cells (but not HeLa cells left alone) displayed MV-H labelling thus indicating that P815.H cells remained stuck on their surface (data not shown).

**Virus infections.** Jurkat cells were infected with either wild-type vaccinia virus (VV-wt) or vaccinia virus recombinant encoding the MV-H protein (VV-H) at 0.25 p.f.u./cell and left at 37 °C (7% CO₂) overnight. Jurkat cells were infected with MV (Hallé strain) at 0.5 p.f.u./cell for 48 h.

In some experiments, L.H cells were infected overnight with either VV-wt or vaccinia virus recombinant encoding the MV-CD46 protein (VV-CD46) at 1 p.f.u./cell. The efficiency of virus infections was monitored by analysis of surface expression of MV-H protein (for VV-H and MV infection of Jurkat cells) and CD46 (for VV-CD46 infection of L.H cells), respectively.

**Antibodies.** Three monoclonal antibodies (MAbs) directed against various CD46 epitopes were used: MC120.6 (Naniche et al., 1992, 1993a), GB24 (Cho et al., 1991) and J4-48 (Immunotech). In some experiments, MC120.6 was used as a biotinylated form followed by an avidin–phycoerythrin conjugate (Biogeneesis) according to the manufacturer's instructions. MV-H protein was revealed by an anti-MV-H antibody, clone 55 (Giraudon & Wild, 1985). FITC-conjugated goat anti-mouse antibody and anti-CD3 antibody coupled to phycoerythrin (IOT3b) were obtained from Immunotech (Marseille, France). Anti-CD63, anti-CD47 and anti-CD14 antibodies were kindly provided by S. Sealand (Lyon, France).

**Results**

Coculture of two cell lines, one expressing CD46 and the other MV-H protein, results in reduced CD46 cell surface labelling

Coculture of murine L.H fibroblast and human Jurkat cells for 16 h decreased surface expression of CD46 in Jurkat cells by more than 70% when compared to that displayed by control Jurkat cells (Fig. 1 a). This shift was very similar to those due to downregulation of CD46 after infection of Jurkat cells with either VV-H (Fig. 1 b) or MV (Fig. 1 e). By contrast, cell surface expression of H was not changed when L.H cells that were cocultured for 16 h with Jurkat cells were examined for MV-H surface labelling (Fig. 1 c). However, a decrease (60–100%) in the expression of MV-H protein at the surface of L.H cells could be seen after their infection with VV-CD46 as compared to VV-wt-infected or uninfected cells (Fig. 1 d).

These results were obtained by using MC120.6 MAb directed against the epitope of CD46 which is involved in MV binding (Naniche et al., 1992) via MV-H/CD46
interaction (Gerlier et al., 1994a). To ensure that the reduced CD46 labelling after Jurkat/L.H coculture was due to CD46 disappearance (i.e. downregulation) from the cell surface rather than to the absence of CD46 labelling by MCI20.6 (if MCI20.6 and MV-H interact with the same CD46 epitope, then the epitope occupied by MV-H would remain inaccessible to MCI20.6), we repeated these experiments using two additional antibodies, J4-48 and GB24, recognizing epitopes different from that recognized by MCI20.6. Indeed, these two antibodies do not block MV-binding (Dörg et al., 1993). Identical results were obtained with all three antibodies (data not shown).

H-mediated CD46 downregulation is specific and reversible

Downregulation of CD46 observed after cell-to-cell contact is specific for CD46-H interaction since it could be seen after Jurkat and L.H coculture but not after their coculture with either LTK- (Fig. 2a) or L.NP cells (Fig. 2b). This downregulation is restricted to CD46 as it was not observed when surface expression of CD3 (Fig. 2c), CD63, CD47 and CD14 (data not shown) were examined under the same experimental conditions.

Downregulation of CD46 cell surface expression required at least 0.5 h contact between Jurkat and L.H cells; the maximum level of downregulation was achieved after 1 h of coincubation (Fig. 3a). It remained unchanged up to 24 h (after 6, 12 and 16 h of coincubation; data not shown). H-mediated CD46 downregulation is reversible. Indeed, in Jurkat cells in which maximal receptor downregulation was achieved by L.H coculture, CD46 surface expression was restored after 24 h, returned to the control level after 48 h (Fig. 3b) and then remained unchanged later (up to 72 h, data not shown).

All experiments presented were performed at equi-cellular ratio of Jurkat/L.H cells. Altering the ratio by increasing the number of L.H cells (up to 10:1) could neither accelerate CD46 downregulation nor increase its maximal level (data not shown).

Both anti-CD46 and anti-H antibodies block H-triggered CD46 downregulation

Preincubation of Jurkat cells with either MCI20.6 or C155 antibodies prior to coculture with L.H cells was sufficient to reduce the maximum CD46 downregulation by 50% (Fig. 4). The observed inhibition could not be attributed to the capacity of MCI20.6 to trigger CD46 downregulation by itself (data not shown). In addition, when Jurkat cells were preincubated with both MCI20.6 and C155 prior to coculture with L.H cells, the observed CD46 downregulation represented only 25% of the downregulation observed in the absence of antibodies (Fig. 4).

Next, we examined the effect of a synthetic oligopeptide (Z-D-Phe-L-Phe-Gly) on H-dependent CD46 downregulation. This peptide has been found to inhibit MV-induced cell fusion but its precise site of action (inhibition of virus attachment by preventing MV-H/CD46 interaction or inhibition of later steps of fusion by inhibiting MV-F interactions with the cell membrane) could not be determined (Richardson & Choppin, 1983). Jurkat cells were preincubated with the oligopeptide (1 μM to 100 μM) or left without treatment prior to coculture (2 h, 37 °C) with L.H cells. No difference in CD46 surface labelling was observed between these experimental conditions (Fig. 4) thus strongly suggesting that the oligopeptide interferes with cell fusion at steps subsequent to MV attachment.

Activators of neither protein kinase C (PKC) nor of protein kinase dependent on cAMP (PKA) trigger CD46 downregulation

To assess whether CD46 could be induced to downregulate by the PKC intermediate, we studied the effect of its activator (Gschwendt et al., 1991), TPA (12-O-tetradecanoylphorbol 13-acetate; Sigma) on surface CD46 expression in Jurkat cells. The CD46 expression remained unchanged even after 1 h treatment of cells with TPA at a concentration as high as 1 μM (Fig. 5). However, the CD3 labelling of the same cells, showed that treatment with 10–30 ng TPA was sufficient to trigger its downregulation (Fig. 5). The TPA solvent DMSO at the highest concentration used (0.01% corresponding to 1 μM concentration of TPA) had no obvious effect on CD46 and CD3 surface labelling.

We next assessed the capacity of PKA activators such as dibutryl cAMP and forskolin to trigger CD46 downregulation. In these experiments, Jurkat cells were first pretreated with 1 mM-3-isobutyl-1-methylxanthine (IBMX; Sigma) for 60 min (37 °C) in order to inhibit endogenous cAMP phosphodiesterase (a cAMP-degrading enzyme). Cells were then divided in different batches and left untreated (control group) or treated for an additional 5, 15, 30 or 60 min with either 1 mM-dibutyryl cAMP (Sigma) or 100 μM-forskolin (Sigma). Drug concentrations used were chosen according to previously reported data about their efficiency at activating adenylcyclase (cAMP-producing enzyme) in Jurkat cells (Bihoreau et al., 1991). No decrease in CD46 surface labelling was observed in treated compared with control Jurkat cell groups (data not shown).
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Fig. 2. Downregulation of CD46 is specific for interaction of H and CD46 molecules (a, b) and is restricted to CD46 (c). Jurkat cells were either left alone (−L.H/−L.TK− in Fig. 2a and −L.H/−L.NP in

Fig. 3. Kinetics of H-dependent CD46 downregulation (a) and its reversibility (b). Jurkat cells were either coincubated with L.H fibroblasts for different time periods or left alone. After indicated times, Jurkat cells were processed for MCI20.6 labelling as described in Methods. For experiments presented in (b) panel, cells were kept in contact with L.H fibroblasts for 16 h, then separated, washed and put in culture for different time periods (24 h, 48 h and 72 h). They were labelled for quantification of CD46 expression as described.

Discussion

In the present paper we report for the first time that interaction between two opposed membranes expressing either CD46 or MV-H protein triggers the rapid and reversible downregulation of CD46 but not H. The downregulation described herein is related to CD46—H interaction because it could be seen only after coculture of CD46+ cells with cells expressing MV-H protein.

Fig. 2b) or coincubated for 16 h with: (a) L.H; (b) LTK−; (c) L.NP fibroblasts. They were then separated from fibroblasts, washed and examined for CD46 surface labelling according to the same experimental protocol as in Fig. 1(a). CD3 surface labelling of the Jurkat cells displays no difference between: (a) Jurkat cells left alone (−L.H/−L.NP); (b) coincubated with L.NP and (c) coincubated with L.H fibroblasts (c).
Moreover, blocking effects of either MCI20.6 (anti-
CD46 MAb) or C155 (anti-H MAb) brought additional
evidence for the involvement of CD46/H interaction in
H-dependent CD46 downregulation. Such a conclusion
was further supported by the synergistic inhibition of
CD46 downregulation by both antibodies. Relevantly,
MCI20.6 inhibition of neither MV-H triggered CD46
downregulation (present study) nor of MV attachment
(Naniche et al., 1992) was complete.

Interaction between CD46 and MV-H protein leads to
a specific downregulation of CD46 since four other
membrane glycoproteins are not affected by this in-
teraction. However, at least two of them (CD3 and
CD14) are able to be downregulated from the cell surface
when an appropriate stimulus is applied. Indeed, CD3
downregulation can be triggered by specific T cell
receptor antibodies, by supra-optimal doses of antigen or
by phorbol esters (present study; Dietrich et al., 1994).
Similarly, CD14 appears to be downregulated from the
cell surface after interaction with an antibody mimicking
the effects of its ligand, lipopolysaccharide (Lee et al.,
1993).

The data presented here demonstrate unequivocally
that interaction between the extracellular domains of
both CD46 and MV-H is necessary and sufficient to
trigger a downregulation of CD46. Indeed, in our
experimental system any putative interaction between
cytosplasmic regions of CD46 and H proteins is prevented
by the fact that they are expressed on the surface of two
distinct cells. In contrast, MV-H downregulation
triggered by H–CD46 interaction could be observed only
when both molecules are expressed on the surface of
the same cell and not after cell-to-cell contact. Therefore,
the mechanism of MV-H-mediated CD46 downregulation
appears to be fundamentally different from that of
CD46-triggered MV-H downregulation. In this latter
case, indeed, it requires interaction between the cyto-
plasmic and/or transmembrane domains of CD46 and
MV-H protein. However, our data do not exclude the
possibility of an eventual modulatory role of CD46
cytosplasmic domain in mediating H-dependent CD46
downregulation. In this regard, it is relevant that it
remains currently unknown whether H binding to CD46
activates signal transduction pathways and whether, if
activated, these pathways interfere with CD46 down-
regulation.

To assess the mechanism of CD46 downregulation, we
asked whether phosphorylation of CD46 can trigger its
downregulation. Indeed, for many receptors, phosphory-
lation of intracytoplasmic domain is often a signal
triggering its downregulation. For example, phosphory-
lation of β2-adrenergic receptor by either β2-adrenergic
receptor kinase or by PKA precedes its downregulation
(Dohlman et al., 1991). Our data obtained with activators
of PKA indicate that this kinase is not involved in
triggering CD46 downregulation. On the other hand, it
has been reported that muscarinic acetylcholine m1
receptor is downregulated subsequent to its phosphory-
lation by either PKA (Lee & Fraser, 1993) or by PKC
(Ross et al., 1988). Similarly, the CD3γ subunit of the T
cell receptor (TCR) is downregulated following its
phosphorylation by PKC (Dietrich et al., 1994). How-
ever, in our experiments, PKC activation by TPA was
unable to trigger the downregulation of CD46 even if (as
has been reported in the same experimental conditions)
TPA treatment results in CD46 phosphorylation (Russel
et al., 1992). Altogether, these data suggest a PKC-
inddependent mechanism of CD46 downregulation.
Similarly, internalization of epidermal growth factor
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Fig. 5. TPA triggers downregulation of CD3 but not of CD46. Jurkat cells were treated for 60 min at 37 °C by TPA in different concentration (0.1, 1, 10, 100, 200, 500 and 1000 nM). Cells were then washed, divided in two batches and processed for CD3 (upper panel) and CD46 (lower panel) labelling as described in Methods. Control value gives the background fluorescence obtained by incubation of cells with avidin-phycoerythrin alone.

(Morrison et al., 1993) and transferrin (Rothenberger et al., 1987) receptors can be dissociated from their phosphorylation by PKC.

What would be the physiological significance of CD46 downregulation after MV infection? Downregulation of CD46 renders cells more susceptible to complement lysis (Schnorr et al., 1995) and might therefore be important for the induction of lymphopenia and virus-associated immunosuppression. In this regard, our results on cell-to-cell contact-induced downregulation of CD46 are consistent with the hypothesis of Yanagi and co-workers (Yanagi et al., 1992) who postulated that the direct contact between MV-infected and non-infected cells plays an essential role in establishment of immunosuppression. Therefore, the comprehension of the molecular basis of H-induced CD46 downregulation is
fundamental for better understanding of measles pathology. Our experimental system provides a simple and attractive model to study it.

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References


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