Three Monoclonal Antibodies Against Measles Virus F Protein Cross-React with Cellular Stress Proteins

HOOSHMAND SHESHBERADARAN* AND ERLING NORBY

Department of Virology, Karolinska Institutet, School of Medicine, Stockholm, Sweden

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In a group of 11 monoclonal antibodies specifically reacting with the measles virus fusion protein, three antibodies also immunoprecipitated other proteins, in particular a 79,000-molecular-weight protein from virus-infected cells. The cross-reacting 79,000-molecular-weight protein was shown to be a virus-induced host stress protein. This protein could be induced by (i) different paramyxoviruses, (ii) heat shock of uninfected HeLa cells, and (iii) 2-deoxyglucose, tunicamycin, or L-canavanine treatment of different mammalian cell lines. Immunofluorescence of stressed HeLa cells localized the cross-reacting host protein(s) mainly in the cytoplasm.

The significance of these results in relation to autoimmune phenomena is discussed.

The presence of autoantibodies in sera from individuals infected with viruses such as hepatitis types A and B, varicella zoster, Epstein-Barr, mumps, or measles is a recognized phenomenon (10, 15, 18, 19, 28). It has been proposed that induction of autoimmune responses by viruses may be caused by shared determinants on molecules normally present on host cells, by alteration of the host immune system, or by the expression or release of "normally sequestered" self antigens (7, 14, 16). The results of this study indicate that a combination of these alternatives may also occur. One class of cellular proteins that are normally either absent or present in very low concentrations are the so-called stress proteins (see reference 25). Such proteins have been shown to accumulate rapidly in avian and mammalian cells in response to a variety of stresses including heat shock, amino acid analogs, certain heavy metals, sulhydryl reagents, inhibitors of glycosylation, and tissue explantation. Recent studies have shown that cellular proteins related or identical to certain stress proteins are also induced by infection with several lytic viruses including simian virus 40 and polyoma virus (13), herpes simplex virus (22), adenovirus (20), Sindbis and vesicular stomatitis virus (8), and paramyxoviruses: Newcastle disease virus (4), parainfluenza type 1, and simian virus 5 (23). In this report, we show that the measles virus fusion (F) component shares an antigenic determinant(s) with at least one virus-induced cellular stress protein.

Measles virus encodes two envelope glycoproteins, hemagglutinin (H) and F; the latter is composed of two disulfide-bonded polypeptides, F1 and F2 (41,000 molecular weight [41K] and 18 to 24K, respectively). Of the previously reported (26) 11 anti-measles F monoclonal antibodies (MAbs), three, 16-AG5, 16-DC9, and 16-EE8, were seen to immunoprecipitate additional bands, in particular a 79K protein (designated c79), besides the F1 polypeptide from [35S]methionine-radioabeled virus-infected cell lysates (Fig. 1). The three MAbs described above will be referred to as anti-F/c79, and the remaining eight will be referred to as anti-F. Similar immunoprecipitation patterns were obtained regardless of the strain of lytically replicating measles virus studied, and in several cell lines persistently infected with measles virus (Lu106 carrier, MaSSPE, and HEpPi; cell lines described in H. Sheshberadaran, E. Norrby, and K. W. Rammohan, Arch. Virol., in press). As the c79 comigrated with the measles H protein (Fig. 2), the possibility that it might represent H protein contamination was investigated by means of a depletion radioimmunoprecipitation assay (RIPA). Lu106 carrier cells were employed in this experiment as they were a rich source of both H and c79, although they accumulated little of the F protein. The results (Fig. 2) indicated that c79 and H were different proteins. Furthermore, unlike the H protein, c79 could not be radiolabeled with 1,6-[3H]-N-acetylglucosamine and 2-[3H]mannose (data not shown). Although not prominent in mock-infected Vero cells, a c79 protein cross-reacting with the anti-F/c79 MAbs could be induced in Vero cells lytically infected with parainfluenza viruses types 1, 2, and 3, mumps, canine distemper, or respiratory syncytial virus as judged by RIPA (selected examples are shown in Fig. 1).

In view of the above-mentioned reports on virus-induced cellular stress proteins, heat-shocked HeLa cells were analyzed for the presence of a c79 protein. Among other proteins, a c79 protein cross-reacting with the anti-F/c79 MAbs was strongly induced by heat shock in HeLa cells (Fig. 3). Further analysis showed that c79 could be induced in HeLa, Vero, and rabbit kidney (RK-13) cell lines with 2-deoxyglucose (Fig. 4) or tunicamycin and in HeLa and Vero cells with L-canavanine (RK-13 cells were not examined for L-canavanine induction). The c79 protein is therefore also a glucose-regulated protein and is probably the same protein as the 78K glucose-regulated protein (GRP-78) induced by parainfluenza type 1, simian virus 5, and Newcastle disease virus reported in previous studies (4, 23). Competition RIPA showed that binding of radiolabeled measles F to anti-F/c79 MAbs could be blocked by unlabeled extracts of stressed HeLa cells (data not shown). Immunofluorescence (IF) of normal HeLa cells with the MAbs showed that the anti-F/c79 MAbs gave diffuse cytoplasmic staining, with a cagelike network surrounding the nucleus (Fig. 5a). This was not surprising inasmuch as small amounts of c79 and some other proteins were immunoprecipitated from normal HeLa cells (Fig. 3 and 4). L-Canavanine-treated HeLa cells showed a marked enhancement in cytoplasmic staining, especially in the perinuclear region (Fig. 5b), whereas 2-deoxyglucose treatment not only resulted in enhanced perinuclear staining (Fig. 5c), but with one of the antibodies, 16-EE8, distinct dot-like intranuclear peripheral staining was obtained (Fig. 5d). It therefore appears that depending on the inducer, more than one cross-reactive protein species can be induced in the

* Corresponding author.
FIG. 1. Induction by different paramyxoviruses of a c79 protein cross-reactive with three measles anti-F MAbs. [35S]methionine-radiolabeled measles (A), parainfluenza type 2 (B), respiratory syncytial (C), and mock-infected (D) Vero cell lysates in RIPA buffer (3% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% aprotinin, 3 mM phenylmethylsulfonyl fluoride, 0.15 M NaCl, 0.01 M Trishydrochloride [pH 7.4]) were immunoprecipitated with the following: anti-measles F MAbs, 16-EE8 (lane 1), 16-DC9 (lane 2), 16-AG5 (lane 3), 19-FF4 (lane 4), 19-HB4 (lane 5), 19-HC4 (lane 6), 19-GD6 (lane 7), 19-FF10 (lane 8), and 9-D810 (lane 9); and polyclonal rabbit anti-parainfluenza type 2 (lane 10) and anti-respiratory syncytial virus (lane 11) sera. Radiolabeling, RIPA, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% acrylamide in A; 12.5% acrylamide in B, C, and D) were performed as described previously (26). Arrowheads indicate positions of molecular weight markers (myosin, 200K; phosphorylase b, 92.5K; bovine serum albumin, 69K; ovalbumin, 46K; and carbonic anhydrase, 30K). Actin is indicated by a.

host cell: cytoplasmic protein(s), which in IF exhibits epitopes reactive with all three anti-F/c79 MAbs, and nuclear protein(s), which in IF exhibits only 16-EE8 reactive epitopes. Data from competitive binding assays with measles virus indicate that the three anti-F/c79 MAbs recognize closely associated or overlapping epitopes (A. Salmi and E. Norrby, unpublished observation). RIPA on 2-deoxyglucose-treated HeLa cells did not reveal any major differences between 16-EE8 and the other two anti-F/c79 MAbs (data not shown), indicating that both cytoplasmic and nuclear cross-reactive proteins are c79 species. However, it should be noted that the partial denaturing conditions in RIPA could reduce or exclude low-affinity reactions. These IF data are not unexpected: using antibodies against GRP-78 purified from membrane fractions in IF studies, Shiu and Pastan (27) have reported that GRP-78 is localized in a granular vesicular network inside the cell resembling the distribution of endoplasmic reticulum. Cell fractionation studies have localized GRP-78 in membrane and cytosol fractions as well as in nuclear fractions (17, 30).

Besides the c79 protein, minor bands, notably those of ca.

FIG. 2. Analysis of c79 and measles H proteins by depletion RIPA. Samples (200 µl) of [35S]methionine-radiolabeled Lu106 carrier cell lysate (in RIPA buffer; see the legend to Fig. 1) were immunoprecipitated three consecutive times with either an anti-H MAb, I-44 (lanes 1 to 3) or the anti-F/c79 MAb, 16-AG5 (lanes 6 to 8). The resultant depleted cell lysate was immunoprecipitated with 16-AG5 (lane 4) or I-44 (lane 9). Lane 5 shows molecular weight markers (see the legend to Fig. 1). Actin is indicated by a. For conditions of radiolabeling and details of the method, Sheshberadar et al., in press.
either 3C79m, 150K, 100K (prominent in heat-shocked HeLa cells [Fig. 3] and occasionally seen as a doublet [Fig. 2]), 52K, 47K, 40 to 41K (not seen in HeLa but prominent in Vero cells [Fig. 4]), and 33K were also immunoprecipitated from virus-infected or otherwise stressed cells. Their amounts, however, were variable between different experiments. It was therefore not possible to assess whether they represented true cross-reacting species or were simply co-precipitated like cellular protein (denoted in figures as a: 43K). Attempts to resolve this issue by the Western blot technique (3) were unsuccessful. The anti-F/c79 MAbs gave a very weak reaction, even with the c79 protein in Western blots. This was not surprising as none of the 11 anti-F MAbs reacted with the measles F protein in immunoblotting, indicating denaturation of the relevant antigenic determinants.

The advent of MAbs has consolidated the concept of "molecular mimicry" by viruses. Recent reports include demonstration of shared epitopes between various host cell components and simian virus 40 large T antigen (5), vaccinia virus H glycopeptide (6), Japanese encephalitis virus non-structural protein (9), measles virus phosphoprotein, and herpes simplex type 1 146K protein (7). In many cases, these cross-reactions involve epitopes on cytoskeleton components. To date, none of the reports of molecular mimicry by viruses have involved inducible host cell components. The observation of shared epitopes between a viral protein and a stress protein greatly extends the arena within which molecular mimicry by viruses may play a role in autoimmune pathology. Since stress proteins can be induced by physical and chemical stresses as can some viruses, previous encounter with a stress protein cross-reactive virus may play a role in the immunopathology of an unrelated virus infection or other nonvirus-associated autoimmune conditions in which the same or partially homologous stress proteins are induced. Of interest in this respect, and of particular relevance to targeting of the immunopathological response, are the observations that eucaryotic cells contain not only different

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FIG. 3. Induction by heat shock of a HeLa cell c79 protein cross-reactive with anti-measles F/c79 MAbs. Medium on confluent HeLa monolayers (9 × 10⁶ cells) grown at 37°C was replaced with 10 ml of medium containing 1/30 of the normal concentration of methionine and 300 μCi of [¹⁰¹]2S)methionine, and the cells were incubated at either 37 or 42°C for 24 h. RIPAs on cell lysates (400 μl per assay) were carried out with the following MAbs: 1-41 (anti-H; lanes 1 and 1′), 16-AC5 (anti-NP; lanes 2 and 2′), 16-DC9 (anti-F/c79; lanes 3 and 3′); 16-EE8 (anti-F/c79; lanes 4 and 4′); and 9-DB10 (anti-F; lanes 5 and 5′). Arrowheads indicate positions of molecular weight markers (see the legend to Fig. 1), and a indicates actin.

FIG. 4. Induction of c79 protein in HeLa (A), RK-13 (B), and Vero (C) cells with 2-deoxyglucose. Medium on confluent cell monolayers was replaced with [¹⁰¹]2S)methionine-containing medium (see the legend to Fig. 3) with no additives (−; lanes 1 to 3) or 10 mM 2-deoxyglucose (+; lanes 1′ to 3′), and cells were reincubated at 37°C for 24 h. RIPAs on cell lysates were carried out with the following measles MAbs: 1-41 (anti-H; lanes 1 and 1′), 16-AG5 (anti-F/c79; lanes 2 and 2′); and 16-DC9 (anti-F/c79; lanes 3 and 3′). Arrowheads indicate positions of molecular weight markers (see the legend to Fig. 1), and a indicates actin.
stress proteins but also a number of genes for related stress proteins, and that different stresses induce overlapping sets of these proteins (25). Furthermore, cells from different organs (11, 12, 29) or at various stages in differentiation (1, 2, 24, 31, 32) preferentially express different sets of stress proteins, and different viruses induce different sets of these proteins (4, 8, 13, 20, 22, 23). In the instance reported in this study, the cross-reacting stress protein (c79) was induced during virus infection. Although simultaneous induction of the cross-reacting host component(s) may not be a necessary condition, such a mechanism may augment breakdown of the tolerance of the host and further potentiate immunopathology of the disease.

It should be emphasized that no information is presently available about the pivotal premise on which the above postulates are based, namely, the capacity of the immune system to react to different stress proteins under "normal" in vivo conditions. Furthermore, no information is available on the induction of mammalian stress proteins in vivo during viral infection or by chemical inducers, natural or otherwise. Finally, caution should be exercised in extrapolation of immunological cross-reactions noted with MAb's. Presently, no direct evidence is available that the examples of molecular mimicry by viruses cited are of significance in vivo. Considering the nature of in vivo antibody response to a single epitope compared with the interactions between one pure clonal antibody to that or related epitopes (16), many such cross-reactions with MAb's may be purely chance events of no significance in autoimmune pathogenesis.

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LITERATURE CITED


FIG. 5. Cross-reactivity of measles anti-F/c79 MAbs with uninfected HeLa cells visualized by indirect IF. Mock-treated (a), L-canavanine-treated (10 mM in arginine-free medium for 10 h; b), and 2-deoxyglucose-treated (12 mM in normal medium for 24 h; c and d) HeLa cells were acetone fixed and reacted with 16-EE8 (a and d) or 16-AG5 (b and c). For details of the method, see reference 21.
autoantibodies against intermediate filaments, one of them cross-reacting with the virus hemagglutinin. J. Immunol. 131:1546–1553.


