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A murine cytomegalovirus-neutralizing monoclonal antibody exhibits autoreactivity and induces tissue damage *in vivo*

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SUMMARY

The autoreactivity of murine cytomegalovirus (MCMV)-neutralizing monoclonal antibody (mAb) AC1 was examined *in vitro* and *in vivo*. Both mAb AC1 and a human antiserum reactive with U1-small nuclear ribonucleoprotein (U1-snRNP) stained uninfected mouse embryo fibroblasts (MEF) in a speckled nuclear pattern and reacted with 70,000 molecular weight (MW) MEF nuclear antigens by immunoblotting, suggesting that mAb AC1 cross-reacted with the 70,000 MW component of U1-snRNP. However, only mAb AC1 cross-reacted with an additional epithelial cytoplasmic autoantigen present in cultured HEp2 cells. On tissue sections from uninfected mice, mAb AC1 predominantly reacted with a component of central and peripheral nervous systems, although cross-reactivity with the stratum spinosum of the skin and the outer sheath of hair follicles was also observed. Immunoblotting revealed that mAb AC1 reacted with phosphorylated epitopes present on a 98,000 MW MCMV structural protein and the 200,000 MW mouse neurofilament protein (NFP). Treatment of uninfected mice with mAb AC1 resulted in a severe interstitial pneumonia with greatly thickened and congested alveolar septa. Severe oedema of the hypodermis and a mild mesangial proliferative glomerulonephritis were also observed. These results demonstrate that a mAb reacting with a MCMV structural phosphoprotein which can protect mice against the dissemination of MCMV, can also promote the development of autoimmune disease. Therefore, the production of such cross-reactive antibodies may be an important mechanism in the development of autoimmunity following viral infection.

INTRODUCTION

Infection with human cytomegalovirus (HCMV) has often been implicated in the induction of immunopathological disease. Sero-epidemiological studies have suggested an association between HCMV infection and diseases with an autoimmune component, such as acute polyneuropathy, acute myocarditis and Sjögren's syndrome. Furthermore, rheumatoid factors and antibodies to nuclear, smooth muscle and erythrocyte antigens have been detected in sera from patients following clinical HCMV infection (reviewed in ref. 1). Although the mechanisms by which HCMV infection may induce these autoantibodies are unknown, a number has been suggested. These include polyclonal B-cell activation, molecular mimicry between viral and host cell epitopes leading to the production of cross-reactive antibodies, incorporation of host cell proteins into the virion during virus assembly, and the fusion of genomic and viral DNA leading to the expression of hybrid viral proteins (reviewed in ref. 1).

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Evidence is accumulating to support a role for cross-reactive antibodies in viral-induced autoimmunity. Infection with a number of viruses results in the induction of antiviral antibodies which are autoreactive and have been implicated in the pathogenesis of various autoimmune diseases. For example, antibodies to measles virus, Coxsackie B4 virus and human immunodeficiency virus (HIV-1) cross-react with myelin basic protein, cardiac myosin and HLA class II molecules, respectively.[2] Furthermore, sequence homology and immunological cross-reactivity between HCMV and the β chain of the HLA-DR transplantation antigen has been reported,[3] while Landini *et al.*[4] have recently described immunological cross-reactivity between a HCMV assembly protein and a host cell membrane antigen. These authors suggested that immune responses to epitopes shared by HCMV and host antigens contributed to the immunopathological sequelae associated with HCMV infection. Additional homologies between HCMV and cellular DNA sequences have also been reported,[5,6] although the immunogenicity of these HCMV products, if expressed during the infectious cycle, is unknown.

Murine cytomegalovirus (MCMV) has proven to be a useful model for the study of the relationship between CMV infection, autoimmunity and disease. In susceptible mouse strains,

MCMV infection has been shown to induce diseases such as myocarditis, pneumonitis and hepatitis,[7-9] each of which has an immunopathological component. Multiple autoantibodies, which persist in the absence of viral antigen, have also been detected in mouse sera following MCMV infection.[10]

We have previously reported[11] that monoclonal antibody (mAb) AC1 both reacts with a neutralizing epitope on an immunodominant 98,000 molecular weight (MW) MCMV structural protein and cross-reacts with a nuclear antigen present in uninfected mouse embryo fibroblasts (MEF). This cross-reactive nuclear pattern is reminiscent of that observed with sera from patients with mixed connective tissue disease (MCTD), in which autoantibodies to the U1-small nuclear ribonucleoprotein (snRNP) complex predominate. We report here further characterization of the cross-reactivity of this mAb, and describe both its *in vitro* reactivity with tissues from normal, uninfected mice and its immunopathological effect *in vivo*.

MATERIALS AND METHODS

Mice

Eight- to 10-week-old, specific pathogen-free, inbred female BALB/c mice were obtained from the Animal Resources Centre, Murdoch University, Western Australia. Mice were maintained under minimal disease conditions and were shown to be free of MCMV, mouse hepatitis virus, Sendai virus and *Mycoplasma pulmonis* by routine serological screening.

Virus

The K181 strain of MCMV[12] was propagated in MEF[13] and used as the source of viral antigen in this study. Virulent MCMV was prepared by salivary gland passage of K181 as described elsewhere.[13]

Antigen preparations

Nuclear and cytoplasmic fractions of MCMV-infected and uninfected MEF were prepared as previously described.[13] Purified MCMV antigen was prepared from the extracellular fluid of MCMV-infected MEF by centrifugation on potassium tartrate gradients.[14] A neurofilament-enriched preparation was produced from the brain stem and spinal cords of normal, uninfected BALB/c mice according to the method of Julien and Mushynski.[15]

Sera and antibodies

Normal mouse serum (NMS) was obtained from uninfected BALB/c mice. MCMV immune serum was obtained from BALB/c mice 9 days after infection with a single dose of virulent MCMV, as previously described.[13] Monoclonal antibodies AC1, EA4 and 2F7 were raised from BALB/c or C57BL/6J mice immunized with purified MCMV, and were of the IgM isotype.[11] Monoclonal antibodies were partially purified from either spent tissue culture supernatant or ascites fluid by ammonium sulphate precipitation.[11] Preparations of mAb AC1 and 2F7 were then standardized to a concentration of 2 mg/ml total protein, according to the method of Bradford.[16] A commercial mAb reacting with a 200,000 MW neurofilament protein (NFP-200) was obtained from Amersham (RPN 1103; Amersham, U.K.). Human anti-U1-snRNP serum from a patient with MCTD was obtained from the Department of Clinical Immunology, Queen Elizabeth II Medical Centre, Nedlands, Western Australia. As this serum was also weakly seropositive for HCMV, the control human serum used in this study was obtained from a healthy, HCMV seropositive individual.

Immunofluorescence

Coverslip cultures of uninfected MEF and HEp2 cells were prepared, acetone fixed and reacted with either mAb AC1, NMS, human anti-U1-snRNP serum or the human negative control serum essentially as previously described.[11] Tissues and organs from uninfected BALB/c mice were either frozen in OCT compound (Miles, Elkhart, IN) or fixed for 16 hr in Bouin's fluid. Fixed tissues were transferred to Tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl, pH 7·6) and processed to composite paraffin blocks. All subsequent procedures were performed at room temperature. Frozen or dewaxed paraffin sections were first incubated with TBS containing 20% (v/v) normal goat serum (NGS) for 45–60 min in order to block non-specific antibody-binding sites. The sections were then incubated for 60 min with either mAb AC1, the NFP-200 mAb or NMS appropriately diluted in TBS/10% (v/v) NGS. Slides were washed for 3×15 min with TBS before the addition of fluorescein-conjugated goat $F(ab')_2$ anti-mouse immunoglobulin antibody (Tago, Burlingame, CA) appropriately diluted in TBS/10% (v/v) NGS and 0·001% (w/v) Evans Blue counterstain. Following incubation with the secondary antibody for 60 min, slides were washed four times with TBS, mounted in buffered glycerol and viewed as previously described.[11]

Enzyme immunoassay (EIA)

The reaction of mAb with MCMV antigens was quantified by EIA performed essentially as described previously,[11] except that gradient-purified MCMV was used as the antigen. An additivity EIA[17] was used to ascertain whether individual mAb bound to distinct antigenic sites on the virus. The dilutions used for each mAb in the additivity EIA corresponded to the lowest concentration at which saturation binding of the antigen was achieved. Individual mAb were considered to react with different antigenic sites if the sum of their individual optical densities (OD) was similar to the OD obtained when the mAb were added simultaneously to the same EIA well (EIA OD were additive). However, if the combined mAb OD was approximately equal to the OD obtained with a single mAb, this suggested that the two mAb competed for the same antigenic region (EIA OD were not additive). All tests were performed in duplicate and the OD averaged. Normal mouse immunoglobulins, prepared by ammonium sulphate precipitation, were used as the negative control.

Gel electrophoresis and immunoblotting

Proteins were electrophoresed under reducing conditions on either 10% or 8% sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE) using a discontinuous buffer system, and the separated polypeptides were transblotted onto nitrocellulose (0·45 μm pore size; BioRad, Richmond, CA) essentially as described previously.[11,13] When required, blotted proteins were first treated with either periodate or alkaline phosphatase (Type VII-S, Sigma P-5521; St Louis, MO), in order to cleave the carbohydrate vincinal hydroxyl groups[18] or to dephosphorylate phosphoproteins,[19] respectively, before immunostaining with antibodies. In general, blots were immunostained as previously described[13] except that nitroblue tetrazolium (Sigma) and

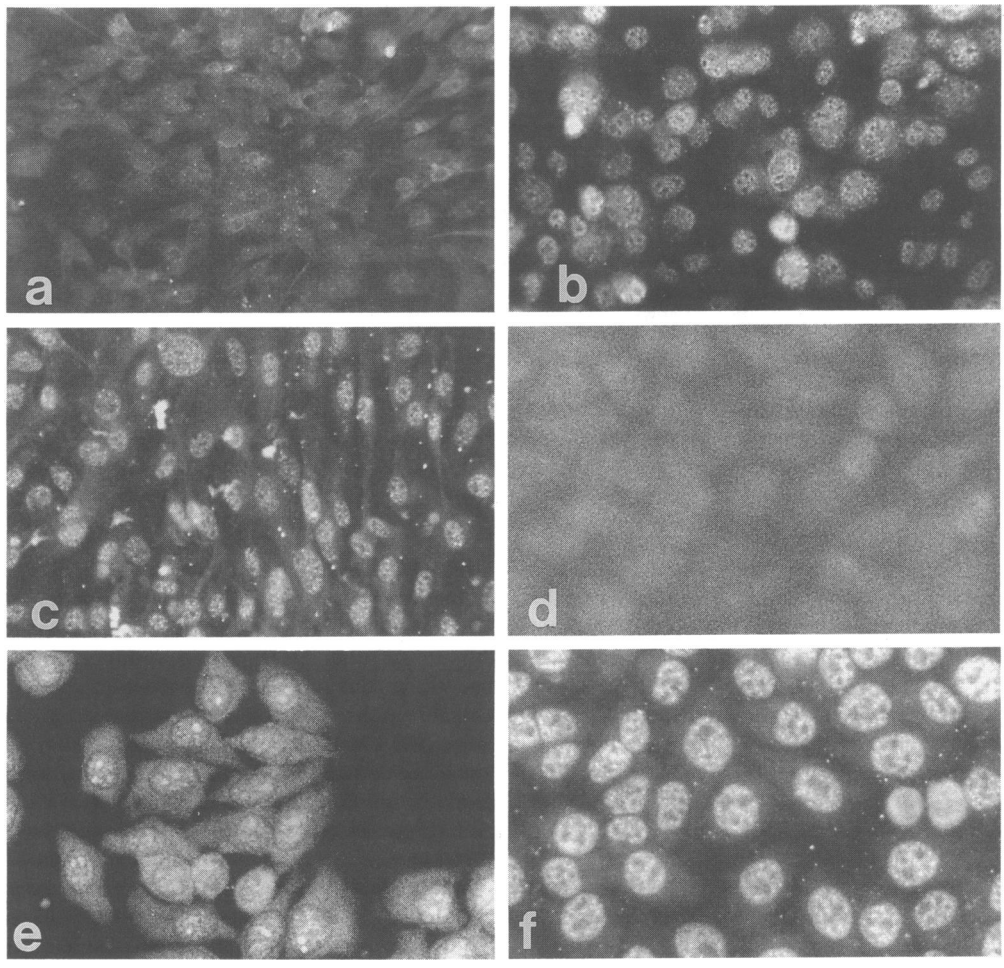

Figure 1. Reaction of NMS (a, d), mAb AC1 (b, e) and human anti-RNP antiserum (c, f) with uninfected MEF (a–c) and uninfected HEp2 cells (d-f) detected by immunofluorescence (× 125).

bromochloroindoyl phosphate (Sigma) were used as the substrate.[20] However, antibodies reacting with bands on blots pretreated with alkaline phosphatase were detected with peroxidase-conjugated goat anti-mouse immunoglobulins (Tago) and 4-chloro-1-naphthol (BioRad).[20]

Treatment of normal, uninfected mice with mAb AC1

Groups of three BALB/c mice that were treated with mAb AC1 either received 0·2 mg of ammonium sulphate-precipitated mAb intraperitoneally (i.p.) three times a week for 7 weeks, or were inoculated i.p. with 1×10^6 hybridoma cells secreting mAb AC1 to induce ascites production as previously described.[11] Mice were killed either 2 days after the final dose of antibody or 10–15 days after inoculation of hybridoma cells, respectively. Any gross pathological change was noted and organs were removed for histological examination.

RESULTS

Immunofluorescence with uninfected cells

Examination of uninfected MEF treated with either mAb AC1 (Fig. 1b) or human anti-U1–snRNP antibodies (Fig. 1c) showed that both reacted strongly with nuclei, resulting in a similar speckled fluorescence pattern. The reaction of both antibodies was confined to the nucleoplasm with no staining of nucleoli. No such reaction was observed with either NMS (Fig. 1a) or the negative control human serum (result not shown). Reaction with uninfected HEp2 epithelial cells showed that while the human anti-U1–snRNP antiserum produced only a speckled nuclear pattern (Fig. 1f), AC1 exhibited both nuclear and cytoplasmic reactivity (Fig. 1e). Neither reaction pattern was observed with NMS (Fig. 1d) or the human negative control serum (result not shown).

Immunoblotting mAb AC1 with MCMV and MEF antigens

The reactivity of AC1 with purified MCMV virions and MEF nuclear antigens was characterized by immunoblotting (Fig. 2). AC1 reacted with 98,000 and 115,000 MW MCMV structural antigens and cross-reacted strongly with a 70,000 MW band present in both uninfected and MCMV-infected MEF nuclei (Fig. 2a). The human anti-U1–snRNP antiserum reacted strongly with an apparently identical 70,000 MW antigen and several low molecular weight (15,000–22,000 MW) antigens from uninfected MEF nuclei, but only reacted weakly with MCMV structural antigens (Fig. 2b). No significant differences were noted between the reaction patterns produced by the

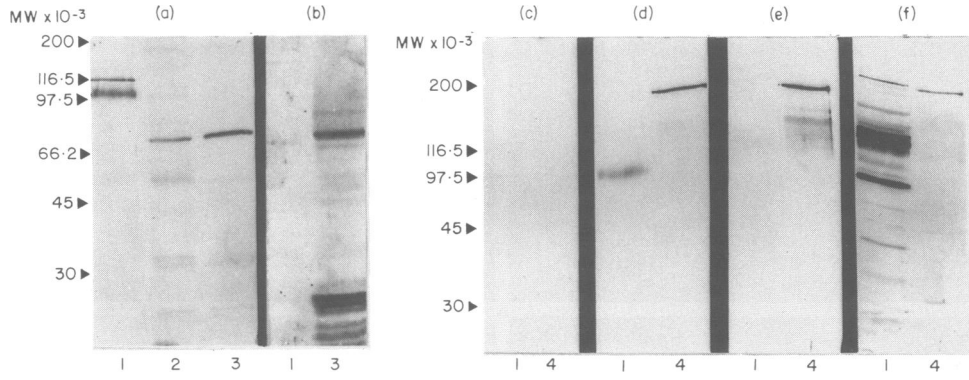

Figure 2. Immunoblotting purified MCMV (lane 1), MCMV-infected (lane 2) and uninfected (lane 3) MEF nuclear antigen preparations, and a mouse neurofilament-enriched preparation (lane 4) with mAb AC1 (a, d); Human U1–snRNP antiserum (b); NMS (c); NFP-200 mAb (e) and day 9 p.i. MCMV immune serum (f). Numbers on the left indicate the positions of standard molecular weight markers.

human U1–snRNP antiserum with either MCMV-infected or uninfected MEF nuclei (results not shown). Consistent with the results observed by immunofluorescence (Fig. 1), AC1 did not react with uninfected MEF cytoplasmic proteins by immunoblotting, however it did react with a 98,000 MW band present in a MCMV-infected MEF cytoplasmic preparation (results not shown).

Reactivity of mAb AC1 and a NFP-200 mAb with uninfected mouse tissues by immunofluorescence

Indirect immunofluorescence on formalin-fixed, paraffin-embedded tissue sections from normal, uninfected mice (Fig. 3) showed that AC1 bound to components of the brain, skin, muscle and mediastinum. Purkinje cell cytoplasm (Fig. 3b) and axons (Fig. 3c) in the brain, the peripheral nerve fibres of muscle (Fig. 3e) and skin (Fig. 3h), and ganglion cells of the carotid body (Fig. 3m) were clearly evident. Treatment of muscle (Fig. 3f) and skin (Fig. 3j) sections with the neurofilament-specific mAb, NFP-200, resulted in an identical reaction pattern to that observed with AC1. In addition, AC1 reacted strongly with the stratum spinosum of the skin (Fig. 3i). NMS did not react with any of the tissues tested (Fig. 3a, d, g, l). When frozen sections of tissue from normal, uninfected mice were treated with AC1, additional reactivity with a component(s) in the outer sheath of hair follicles was observed (Fig. 3k). AC1 did not react with formalin-fixed or frozen tissue sections from the liver, spleen, kidney or lungs (results not shown).

Reactivity of mAb AC1 with neurofilament proteins and characterization of reactive epitope by immunoblotting

The identical immunofluorescent reaction patterns observed for AC1 and the NFP-200 mAb (Fig. 3) suggested that AC1 cross-reacted with a neurofilament protein. Immunoblotting demonstrated that while both mAb reacted with a 200,000 MW band present in a neurofilament-enriched preparation, only AC1 reacted with MCMV antigens (Fig. 2d, e), suggesting that these mAb recognized different epitopes present on the neurofilament protein. In addition, MCMV immune serum obtained from mice 9 days post-infection (p.i.), when autoantibody titres in MCMV-infected mice were maximal, reacted with a number of MCMV antigens and cross-reacted with a 200,000 MW neurofilament component (Fig. 2f). NMS did not react with either viral or neurofilament proteins (Fig. 2c). In order to investigate the nature of the AC1-reactive epitope, blotted MCMV and neurofilament antigens were pretreated with either periodate or alkaline phosphatase before reaction with MCMV-neutralizing mAb AC1 and 2F7 (Fig. 4). Monoclonal antibody 2F7 was included as it appeared to react with the same MCMV antigen as AC1 by immunoblotting (Fig. 4, lane 1). In addition, testing by additivity EIA (Table 1) demonstrated competition between AC1 and 2F7, as the EIA OD obtained when AC1 and 2F7 were simultaneously reacted with MCMV was less than the EIA OD obtained when AC1 was reacted alone. This was in contrast to EA4, which did not appear to compete with AC1, as its combined OD was greater than the theoretical sum of the individual OD. Pretreatment with periodate significantly reduced, but did not eliminate, the reactivity of both AC1 and 2F7 to the 98,000 MW MCMV antigen (Fig. 4, lanes 2–5). In contrast, pretreatment with alkaline phosphatase completely abolished the reactivity of AC1 but did not affect the reactivity of 2F7 with the 98,000 MW MCMV antigen (Fig. 4, lanes 6–9). Similarly, pretreatment of the neurofilament preparation with alkaline phosphatase completely abolished the reactivity of AC1 and day 9 MCMV immune serum (results not shown).

Tissue damage in uninfected mice treated with mAb AC1

As this mAb showed extensive reactivity with normal mouse tissues *in vitro*, normal, uninfected BALB/c mice were treated with AC1 in order to ascertain its *in vivo* effect. All mice which received AC1 developed both lethargy and a marked ruffling of fur. While no gross pathological changes were evident, histological examination of tissues and organs from these mice revealed significant lesions in the lungs, skin and kidneys of those inoculated with AC1-secreting hybridoma cells, or those which received regular injections of purified AC1. Generally, the lesions observed were more severe in those animals which received the antibody-secreting hybridoma cells.

Lungs taken from uninfected mice that either received purified AC1 or were inoculated with AC1-secreting hybridoma cells, displayed a severe interstitial pneumonia (Fig. 5a). The alveolar septa were greatly thickened as granulocytes, macro-

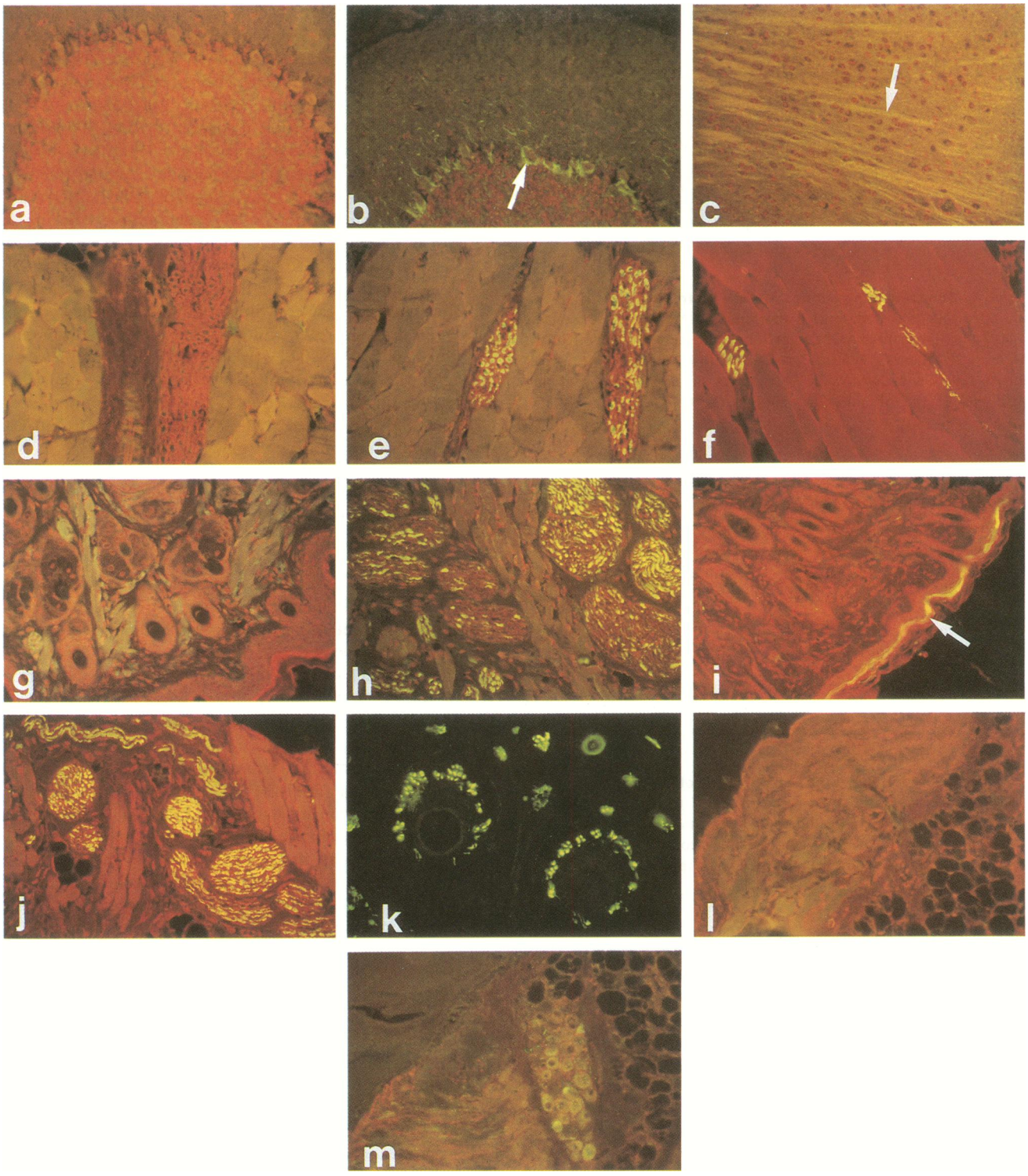

Figure 3. Reaction of NMS (a, d, g, l), mAb AC1 (b, c, e, h, i, k, m) and NFP-200 mAb (f, j) with tissue sections prepared from normal, uninfected mice, detected by indirect immunofluorescence (× 175). Tissue sections were prepared from the brain (a, b, cerebellum; c, white matter), muscle (d–f), skin (g–k; h, j and k, hypodermis) and mediastinum/carotid body (l, m). Arrows indicate reaction with the cytoplasm of Purkinje cells (b), axons (c) and the stratum spinosum of the skin (i). Sections were either prepared frozen (k) or first fixed in Bouin's fluid then paraffin embedded (a–j, l, m).

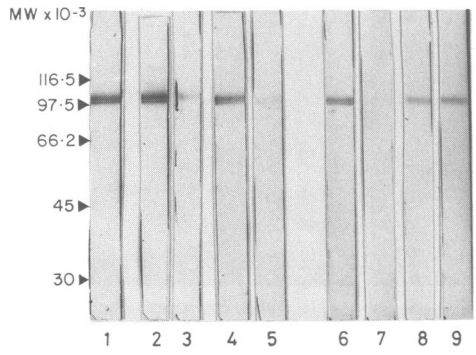

Figure 4. Immunoblotting purified MCMV (untreated, lanes 1, 2, 4, 6 and 8; periodate-treated, lanes 3 and 5; alkaline phosphatase-treated, lanes 7 and 9) with mAb AC1 and 2F7. Lane 1, reaction with both mAb AC1 and 2F7. Lanes 2, 3, 6 and 7, reaction with mAb AC1. Lanes 4, 5, 8 and 9, reaction with mAb 2F7. Numbers on the left indicate the positions of standard molecular weight markers.

Table 1. Reactivity of MCMV mAb with MCMV antigens compared by the additivity EIA[17]

Monoclonal antibodies	OD_{492nm}*	Theoretical sum of OD†
AC1	1·00	—
2F7	0·70	—
EA4	0·71	—
AC1 + 2F7	0·84	1·70
AC1 + EA4	1·95	1·71

* OD subtracted for background.

† The theoretical sum is the sum of the OD obtained for each mAb alone. Monoclonal antibodies were considered to react with distinct antigenic sites if the combined mAb OD was similar to the theoretical sum. Conversely, mAb were considered to compete for the same antigenic region if the combined mAb OD was approximately equal to the single mAb OD.

phages and extravasated erythrocytes accumulated in the interstitium (Fig. 5b). In addition, the septal vasculature was congested. Severe oedema of the hypodermis was observed in the skin of AC1-treated mice (Fig. 5c, arrowheads). The venules near the base of the hair follicles were congested (Fig. 5c, arrow) while macrophages had accumulated in the paravenular regions (Fig. 5d, arrow). The kidneys exhibited a mesangial proliferative glomerulonephritis with granulocytes marginating in the capillary loops (result not shown). The pathological changes observed in the kidney, while significant, were relatively minor compared with the pathology in either the lungs or the skin. No other significant lesions were detected in the tissues of mice treated with AC1.

DISCUSSION

We have previously reported[11] that the neutralization titre of AC1 for MCMV *in vitro* was comparable to that of hyperimmune anti-MCMV serum, and that this mAb protected mice from lethal MCMV infection *in vivo*.[21] AC1 reacts with both 98,000 and 115,000 MW MCMV structural proteins. However, while reaction with the 98,000 MW MCMV protein is observed in all MCMV preparations, reaction with the 115,000 MW protein is observed in only some purified MCMV preparations. Although the reasons for this variability are unknown, it could reflect variable incorporation of a larger (115,000 MW) precursor protein into virions. Treatment of the 98,000 MW MCMV protein with either phosphatase or periodate demonstrated that the AC1-reactive epitope was phosphorylated and possibly glycosylated. Furthermore, ^{14}C glucosamine labelling of MCMV proteins also suggested that the AC1-reactive MCMV proteins were glycosylated (results not shown). In contrast, 2F7, which appeared to bind to the same 98,000 MW MCMV structural protein, reacted with a non-phosphorylated epitope. Since 2F7 does not react with uninfected MEF,[11] it is possible that this 98,000 MW MCMV structural protein contains at least two highly neutralizing epitopes, with only the phosphorylated epitope eliciting the production of autoreactive antibodies.

This study confirmed that AC1 and a human anti-U1-snRNP antiserum produce virtually identical nuclear staining patterns on MEF and demonstrated that both reacted with an identically sized 70,000 MW antigen present in both MCMV-infected and uninfected MEF nuclei. This confirmed previous results[11] where both AC1 and serum from a patient with MCTD reacted with a 70,000 MW component of a ribonuclear extract which seemed likely to be the 70,000 MW component of U1-snRNP.[22] The additional low molecular weight nuclear antigens detected by the anti-U1-snRNP antiserum may represent the 22,000 MW 'C' component of the U1-snRNP complex, in addition to smaller proteolytic degradation products formed during nuclei purification. Although weak reactivity between MCMV antigens and the anti-U1-snRNP antiserum was observed, this may not reflect cross-reactivity of the anti-U1-snRNP antibodies as this serum was obtained from a patient weakly seropositive for HCMV.

Attempts to confirm the identity of the 70,000 MW nuclear antigen through the use of a recombinant p70 U1-snRNP[23] have so far resulted in only weak, inconclusive reactivity with AC1 (results not shown). Since AC1 recognizes a phosphorylated epitope, these results may reflect insufficient phosphorylation of this protein in *Escherichia coli* cells. The recombinant p70 U1-snRNP may therefore require additional phosphorylation *in vitro* before any firm conclusions can be reached concerning the reactivity of AC1 with p70 U1-snRNP. This would not be the first example of molecular mimicry between a viral protein and p70 U1-snRNP, as a retroviral p30gag protein has been found to have both sequence and immunological cross-reactivity with p70 U1-snRNP.[24] Indeed, on the basis of this cross-reactivity it was suggested that molecular mimicry at the level of a single epitope could be an initiating event in autoimmunity.[24]

In contrast to the results obtained with fibroblasts, immunofluorescence with uninfected epithelial cells suggested that, in addition to the ubiquitous 70,000 MW nuclear antigen, AC1 cross-reacted with a cytoplasmic antigen that was present only in epithelial cells. Therefore, it appeared that AC1 cross-reacted with more than one autoantigen. Other multi-specific anti-viral antibodies have also been reported. For example, monospecific antibodies purified with a synthetic peptide corresponding to a region on the Epstein-Barr virus nuclear antigen-1, exhibited multiple autoreactivity with homologous epitopes present on keratin, actin and collagen.[25]

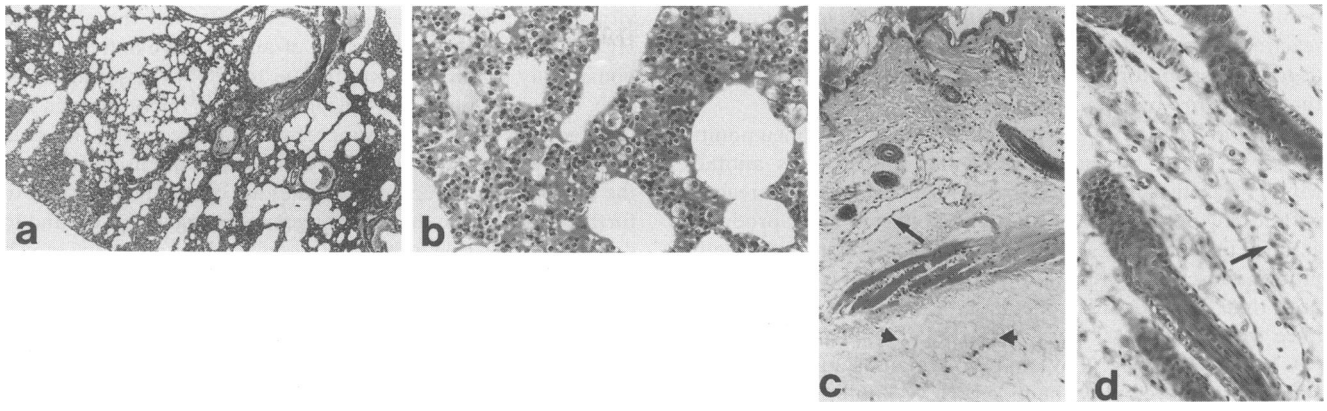

Figure 5. Histological examination of organs taken from normal, uninfected mice treated with mAb AC1. Significant tissue damage was observed in the lungs (a, b) and skin (c, d). Magnification ×20 (a), ×80 (c), ×200 (b) and ×320 (d). Arrows indicate congestion of venules near the base of the hair follicles (c) and the accumulation of macrophages in the paravenular regions (d). Arrowheads indicate areas of severe hypodermal oedema (c).

Subsequently, AC1 was reacted *in vitro* with tissue sections from normal, uninfected mice, in an attempt to determine the full extent of its autoreactivity. AC1 reacted predominantly with central and peripheral neural tissues on these sections. Antigenic cross-reactivity between neural components and other infectious agents has been noted previously.[26] In addition, sera from BALB/c mice infected with MCMV have been found previously to react strongly with peripheral neural tissues.[10] The identical reaction observed with an anti-NFP mAb suggested that AC1 cross-reacted with a NFP. While neurofilaments are generally most abundant in axons,[27] NFP not assembled in the form of neurofilaments are also present in Purkinje cell bodies.[28] Neurofilaments consist of three proteins[27] and the use of a murine neurofilament preparation, in conjunction with the NFP-200 mAb, confirmed that AC1 cross-reacted with a phosphorylated epitope present on the 200,000 MW component of the neurofilament triplet. In addition, antibodies from MCMV-infected mice also cross-reacted strongly with a phosphorylated 200,000 MW NFP epitope and, as phosphorylation of the 200,000 MW NFP only occurs within a defined six amino acid sequence,[29] significant levels of antibodies with reactivities similar to AC1 may be elicited during MCMV infection.

Extending the results observed with HEp2 cells, the use of skin sections revealed that AC1 cross-reacted with some, but not all, epithelial cell types. While this cross-reactive epithelial antigen could not be detected by immunoblotting HEp2 cytoplasmic extracts (results not shown), it could be a cytokeratin as cytoplasmic epithelial cell components such as cytokeratins differ in their polypeptide composition not only between different epithelial strata, but also between different lateral domains of the epidermis.[30] In addition, immunological cross-reactivity between cytokeratins, neurofilaments and ribonucleoprotein has been noted.[31,32]

It has been demonstrated that antibodies to a number of autoantigens can produce pathological changes reminiscent of autoimmune disease. For example, mice receiving an anti-nicotinic acetylcholine receptor (AChR) mAb, either as a purified antibody preparation or by direct inoculation of ascites-producing hybridoma cells, developed severe tissue damage characteristic of myasthenia gravis.[33] In addition, the reported sharing of antigenic determinants between AChR and several Gram-negative bacteria[34] suggests that a humoral immune response to an infectious agent could result in tissue damage characteristic of autoimmune disease. However, the pathological significance of IgM autoantibodies in autoimmune disease is uncertain since they are often regarded as low-affinity, multi-specific antibodies that do not cause tissue damage.[35] This does not appear to be so for AC1, as only restricted reactivity with tissue antigens was noted by immunofluorescence or immunoblotting, and it caused significant tissue damage in normal, uninfected mice. In addition, high circulating levels of IgM autoantibodies to autoantigens such as peripheral neural[36] and nuclear[37] components are often observed in patients with autoimmune disease.

The role of autoantibodies reacting with intracellular antigens, in the pathology of autoimmune disease, is currently unknown; however, it has recently been shown that anti-RNP and anti-DNA antibodies from patients with MCTD and systemic lupus erythematosus, respectively, can enter viable cells and disrupt nuclear function.[38,39]

Treatment of normal, uninfected mice with AC1 resulted in tissue damage reminiscent of that observed in patients with early MCTD. In both situations, oedema and interstitial lymphocytic infiltrates were noted in the skin and lungs, respectively. Also, as in early MCTD,[40] the lungs of AC1 treated mice appeared to be more susceptible to damage than the kidneys, where only minor changes were observed. While the tissue damage observed in the skin of AC1 treated mice may have been due to the direct binding of this mAb to the stratum spinosum and hair follicles, as previously observed on tissue sections, no such binding was observed on either fixed or frozen sections of lung and kidney (results not shown). This suggested that the tissue damage observed in both the lungs and kidneys was most likely a secondary effect of the antibody, perhaps due to immune complex deposition. The development of interstitial pneumonitis following immune complex deposition and the activation of complement has been demonstrated previously.[41] Indeed, most patients with classical MCTD have circulating immune complexes,[42] and it has been postulated that the increased susceptibility to injury of the lungs of patients with MCTD, when compared with their kidneys, may be due to the different nature of the circulating immune complexes in MCTD. However, interstitial pneumonitis similar to that observed in AC1-treated mice can also be observed in animals given anti-lung anti-

bodies.[43] Therefore, it cannot be discounted that lung antigen(s) reacting with AC1 *in vivo* may have been either partially degraded, or conformationally altered during processing, such that no *in vitro* reaction was observed.

The ability of AC1 to cause interstitial pneumonitis appeared to be characteristic of this antibody as another MCMV-reactive mAb, designated 4D8, which cross-reacted with a lung component of normal, uninfected mice, produced extreme tissue damage, but failed to induce interstitial pneumonitis (manuscript in preparation). In addition, it is interesting to note that the interstitial pneumonitis observed in uninfected mice treated with AC1 was very similar to that observed in MCMV-infected mice that had been immunosuppressed with a single dose of cyclophosphamide.[44] HCMV infection of immunosuppressed renal and bone marrow transplant patients often results in a severe interstitial pneumonitis that is a significant cause of morbidity and mortality in these patients.[45] It has been suggested[8,44] that CMV interstitial pneumonitis is immunologically mediated, rather than due to direct viral damage of the lungs, as a host immune response is required for induction of such lesions. Grundy *et al.*[8] further suggested that the pneumonitis was due to the uncontrolled recruitment of T cells recognizing a CMV-encoded non-structural protein in the lung. This conclusion was based on previous findings that non-structural CMV proteins are the major targets of cytotoxic T cells[46] and that antiviral drugs which prevent viral DNA replication, a prerequisite for structural gene expression, had no effect on the development of interstitial pneumonitis.[47] However, the results presented here show that interstitial pneumonitis can be induced in mice by antibody to a MCMV structural protein, in the complete absence of viral antigen. Therefore, an alternative mechanism for the induction of CMV-mediated pneumonitis may be the uncontrolled recruitment of T cells recognizing a lung antigen that is either exposed by, or modified through the binding of, autoreactive antibody elicited by a CMV structural protein. This hypothesis is supported by previous observations[44,48] that MCMV-induced pneumonitis occurs 7–10 days post-infection, which correlates well with the appearance of autoantibodies following MCMV infection.[10]

The results presented in this report demonstrate that a MCMV-neutralizing mAb, reactive with a MCMV structural protein, can cause significant immune damage *in vivo*. As previous research has shown that MCMV-infected mice produce antibody to a MCMV protein with the same apparent molecular weight as that reacting with AC1,[13] then significant antibody levels of this specificity may be elicited in response to MCMV infection. Furthermore, MCMV infection can elicit MCMV-reactive antibodies which cross-react with cardiac myosin and induce myocarditis *in vivo*.[49] Together, these results suggest that molecular mimicry may play a significant role in the induction of autoimmune disease by CMV. However, the initiation of autoimmune disease through the production of autoreactive antibodies may be genetically linked since BALB/c mice produce high autoantibody titres following MCMV infection, while C3H mice produce only low levels of such antibodies.[10]

While the relevance of these results to HCMV infection is not known directly, preliminary experiments have shown that AC1 reacts with a 98,000 MW protein present in HCMV-infected cells (results not shown). This, combined with the fact that several HCMV proteins share immunological cross-reactivity with human autoantigens,[3,4] suggests that the ability of HCMV to elicit the production of autoreactive antiviral antibodies may significantly contribute to HCMV-associated disease. These findings may be of particular importance in the management of immunosuppressed graft recipients infected with HCMV, where anti-HCMV antibodies are used to reduce the level of virus infection.[50] Studies are currently underway to further characterize the autoantigens reacting with AC1 and to determine their role in MCMV-associated autoimmune sequelae.

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