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INVOLVEMENT OF MICROTUBULES AND THE MICROTUBULE-ASSOCIATED PROTEIN TAU IN TRAFFICKING OF JHM VIRUS AND COMPONENTS WITHIN NEURONS*

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INTRODUCTION

The neurotropic coronavirus JHM (JHMV) is capable of inducing various forms of CNS disease in rodents, ranging from an acute encephalomyelitis to a delayed onset demyelination[1,2,3]. In rats, during the early stages of the disease process, neurons become cellular targets[3,4]. When introduced by intranasal inoculation, JHMV can invade the CNS of mice and rats by spreading along the olfactory neurons[5, 6, 7, 8]. Virus spread was shown to occur by the transneuronal route[5,8]. Subsequent spread within the CNS seems to involve specific neuronal populations and tracts[6, 8]. In particular, in Wistar Furth rats, Purkinje and hippocampal neurons are extensively involved[3,4]. Moreover, neurons have been shown to provide a repository site where both RNA and virions can persist for prolonged periods[9, 10, 11]. Recently, Pasick *et al.*, (1994) demonstrated that trafficking of virus materials within neurons occurs asymmetrically along somatodendritic and axonal processes, and appears to be dependent on the integrity of the microtubular network, as evident from analyses by light and electron microscopy which revealed that JHMV nucleocapsids (N) are closely associated with microtubules[12, 13]. This report seeks to define, more directly, by immunoprecipitation and immunoblotting, interactions between N and the microtubular arrays.

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RESULTS AND DISCUSSION

Previous work showed that neurons become infected early during establishment of the CNS disease by JHMV[8, 10, 11]. The predilection of N nucleoprotein for associating with neuronal microtubules was shown previously[12, 13]. This is evident in primary explants of rat hippocampal neurons infected for 24 hours with JHMV, then processed for electron microscopy. As illustrated in Figure 1, the dense granular nucleocapsid material is juxtaposed to microtubules, an observation consistent with previous findings[12, 13] on the colocalization of microtubules and nucleocapsid and determined by means of confocal microscopy following dual antibody marking with immunofluorescence (data not shown).

To explain the observed colocalization of N with microtubules, the database on microtubule-associated cellular proteins was searched for sequence homology which may exist with N. In fact, the microtubule-associated protein tau was found to possess a significant sequence match with N, where an overall 20% identity and 42% similarity was uncovered. It is significant that the closest homology lies within the microtubule-binding domain of the tau sequence which encodes a 31% identity and 54% similarity with N[12].

The phosphoprotein tau which has an mRNA generated by alternative splicing is developmentally regulated. Tau is differentially phosphorylated by several kinases and in its dephosphorylated state acts to stimulate formation of microtubule bundles[14]. Being a microtubule-associated protein, tau interacts with tubulin by a 18 amino acid repeat that constitutes the microtubule-binding domain[16]. Within neurons, tau is localized primarily to axons[15]. Considerable attention has been given to tau as a major pathologic feature in

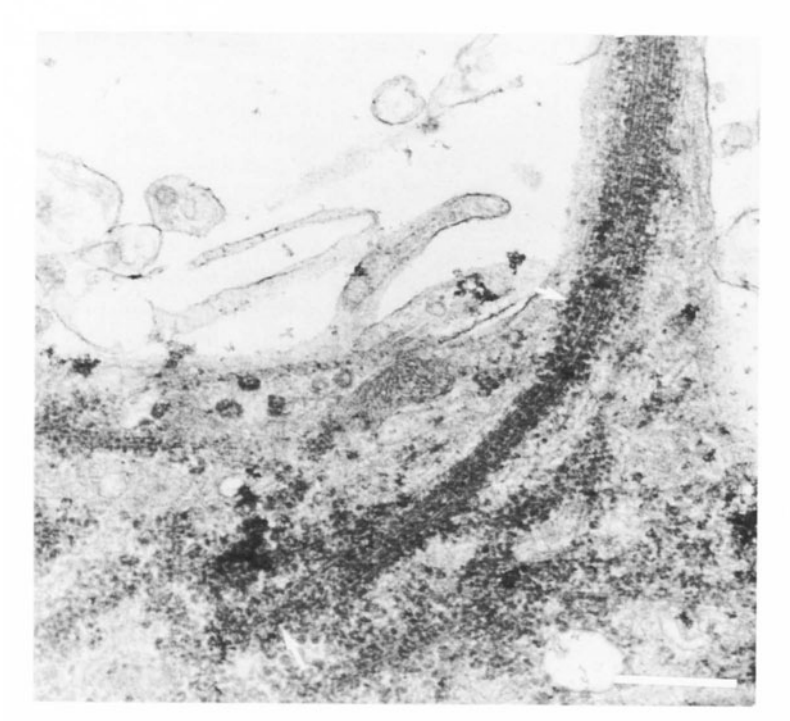

Figure 1. Electron microscopy of a portion of JHMV-infected neuronal cells from a telencephalic explant culture Arrows indicate a close association between the dense, granular nucleocapsid material and the microtubular network Magnification (Bar = 0 5μM) (from [13])

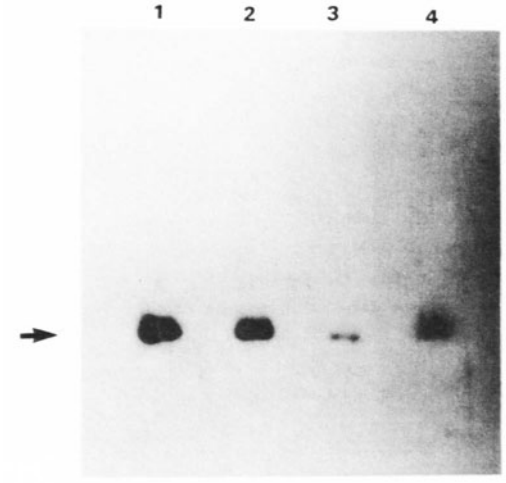

Figure 2. Immunoblot demonstrating cross reactivity between the N protein with anti-tau antibodies. Purified N was probed with two MAbs against N (lanes 1 and 2) or with MAb and polyclonal antibodies against tau (lanes 3 and 4) (from [12])

Alzheimer's disease because it self-assembles and is an integral component of the paired helical filaments constituting neurofibrillary tangles[17].

To determine whether N and tau are immunologically cross reactive, purified N was transferred to nitrocellulose and the blots were probed with anti-N antibodies as controls and both anti-tau monoclonal and polyclonal antibodies. It is evident from Figure 2, that both monoclonal (lane 3) and polyclonal (lane 4) anti-tau antibodies recognized purified N in an immunoblot assay albeit the monoclonal reacted with a lesser intensity.

To analyze the significance of the above findings further, immunoprecipitation and Western blotting were used to assess the interaction of with the microtubular protein. The OBL-21 is a neuronal cell line originating from olfactory neurons of CD.1 mice, which was immortalized by the myc gene of a replication defective avian retrovirus[18]. Following infection for 24 hours with JHMV, OBL-21 cell extracts were reacted with antibodies against tau, tubulin and N. The immunoprecipitates were subjected to SDS-PAGE and the resulting immunoblot probed with anti-N antibodies. As shown in lane 3 of Figure 3, anti-tau antibodies formed a precipitate with N This finding supports the presumption that amino acid sequence relatedness between N and tau is also one of immunological identity at the epitope(s) binding anti-tau antibodies. The ability of anti-tubulin antibodies to bring down N in the immunoprecipitates although in small amount (Figure 3, lane 2) indicates a reactivity with preexisting complexes with N and tubulin, which are established during infection, as

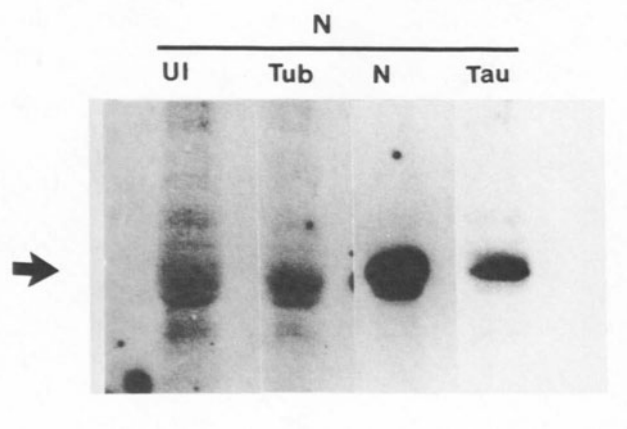

Figure 3. Immunoblot demonstrating the ability of antibodies against tau and tubulin to form immunoprecipitates with N present in JHMV-infected OBL-21 neuronal cells. JHMV-infected cell lysates were mixed with pre-immune serum (lane 1), anti-tubulin (lane 2), anti-N (lane 3) and anti-tau (lane 4) antibodies Immunoprecipitates were separated by SDS-PAGE then probed in immunoblots with anti- N antibodies

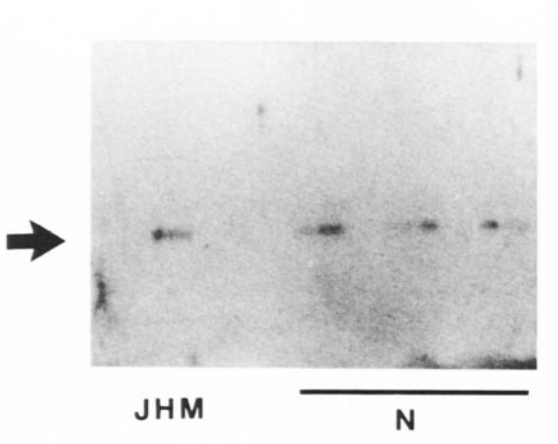

Figure 4. Blot overlay demonstrating the direct interaction between tubulin and N in a nitrocellulose matrix. Purified JHM virions (lane 1) and purified N protein (lanes 3-5) were transferred to nitrocellulose sheets and then purified tubulin was added. Following binding in buffer solution, the attached tubulin was detected by means of anti-tubulin antibodies.

suggested by the example in Figure 1. As a direct test of a specific interaction between N and tubulin, we performed a blot overlay according to procedures previously described[19]. For this we used N isolated and purified from cells and N existing in isolated JHM virions. The material was separated by SDS-PAGE, then transferred onto nitrocellulose membranes. The blots were exposed overnight to purified tubulin (and bovine serum albumin as a competitor) to allow specific tubulin attachment. The complexes formed were then subjected to Western blotting using anti-tubulin antibodies. As evident from Figure 4, the added tubulin appears to bind to the purified form of N (Figure 4, lanes 3-5) and to the N in virions (Figure 4, lane 1). These findings support the view that N is attached to tubulin inside the cell at the amino acid sequence which is homologous with that encoded by the microtubule-binding domain of tau.

In summary, JHMV nucleocapsids are closely associated with microtubules within neurites. The virus nucleocapsid protein (N) has a capacity to bind in vitro to purified tubulin. and evidently also inside infected cells. The existence of both an amino acid sequence and immunological relatedness between N and the microtubule-binding motif of tau suggests that there is a mimicry between the association of N and tau with microtubules .

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