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Neurofilament expression in human T lymphocytes

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SUMMARY

The expression of intermediate filaments in normal cells is mainly determined by their embryonal developmental origin. Flow cytometry using monoclonal antibody RT97 demonstrated that neurofilament was detectable in the human HuT 78 T-cell line and on resting T lymphocytes. Expression was greatly increased on lymphocytes activated for 3 days with phorbol ester. Western blotting confirmed the presence of the 200,000 MW form of neurofilament in T lymphocytes. Stimulation of peripheral blood T cells with phorbol myristate acetate (PMA) or with anti-CD3 monoclonal antibodies resulted in a marked increase in detection of phosphorylated neurofilament on Western blotting. Stimulation of HuT 78 cells with anti-LFA-1 resulted in redistribution of neurofilament from a perinuclear spheroid core into dendritic processes. These data indicate that T cells activated through the T-cell receptor associated complex express an intermediate filament usually associated with neurally derived cells. The finding that neurofilament expression and organization are regulated by T-cell surface molecules suggests a role for this intermediate filament in T-cell function.

Most mammalian cells possess a cytoskeleton containing elements from three filamentous networks, microtubules, microfilaments and intermediate filaments. The expression of intermediate filaments appears to correlate largely with the embryonal origin of the cell.[1] Neurofilaments are the dominant intermediate filament expressed in neuronal cells and comprise three proteins with differing molecular weights categorized as high (200,000, H), medium (160,000, M) and low (68,000, L).[2] While the function of neurofilament is not fully understood, it is felt that it provides a specific support for the developing neurites and in the maintenance of neuronal calibre by the formation of filamentous cross-bridges.[3] We have previously demonstrated that human HuT 78 T cells undergo cytoskeletal reorganization when stimulated with antibodies to LFA-1 or with phorbol myristate acetate (PMA).[4] This cytoskeletal rearrangement results in the production of dendritic processes which may extend for up to 10 times the diameter of the cell soma. Thus, we felt that neurofilament might be an important component in the maintenance of dendritic processes in HuT 78 cells. Recently, it has been reported that neurofilament constitutes a component of the cytoskeleton in thymomas derived from thymic epithelial cells.[5] Our data now suggest that neurofilament may be a constituent of the cytoskeleton not only in the central nervous system (CNS) but also in the HuT 78 cell line and in circulating T lymphocytes.

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HuT 78 was obtained from American Type Culture Collection (ATCC, Rockville, MD) and the subclone HuT 78/2 derived as previously described.[4] Peripheral blood T cells were obtained by venesection followed by Ficoll–Hypaque sedimentation. Cells were either studied in the resting state or following preactivation for 72 hr with either anti-CD3 (OKT3 used as a supernatant) or PMA 25 ng/ml in RPMI-1640 containing 10% foetal calf serum.

We examined neurofilament expression in peripheral blood lymphocytes by intracellular staining and flow cytometry. Briefly peripheral blood lymphocytes were activated for 3 days with PMA, 25 ng/ml, washed, permeabilized with Triton-X and neurofilament expression studied using monoclonal antibody RT97 directed against phosphorylated neurofilament 200[6] (a kind gift of Professor B. Anderton, Institute of Psychiatry, London, U.K.) and indirect immunofluorescence. Anti-murine IE was used as an isotype control. As can be seen (Fig. 1), low level neurofilament expression is detectable in virtually all resting lymphocytes. Activation for 3 days in PMA resulted in significantly enhanced expression of neurofilament (approximately fivefold increase in mean fluorescence intensity) which was present in all lymphocytes.

Immunoblotting studies were performed on Triton insoluble cytoskeletal preparations using monoclonal antibody RT97 to confirm the molecular weight of the species identified. These data clearly demonstrated the presence of immunoreactive neurofilament with a molecular weight of 200,000 in HuT 78 cells (Fig. 2). The neurofilament preparation was free of membrane components as determined by the non-detection of

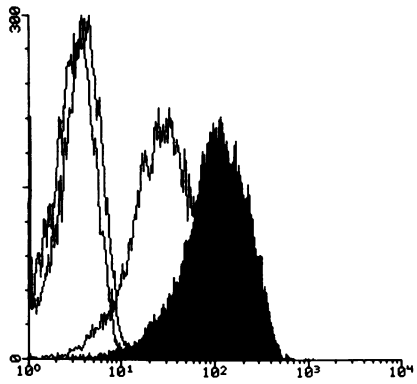

Figure 1. Expression of neurofilament detected by flow cytometry with antibody RT97. The four profiles demonstrated are from left to right: (1) resting T cells immunostained with anti-murine IE; (2) activated T cells immunostained with anti-murine IE; (3) resting T cells immunostained with anti-neurofilament RT97; (4) activated T cells immunostained with anti-neurofilament RT97 (shaded plot). Representative of three experiments.

LFA-1 by immunoblotting with anti-LFA-1 (SPV-L7; Monosan, Sanbio bv, Uden, The Netherlands) previously used to detect LFA-1 on membrane preparations from HuT 78 by Western blotting. Resting T cells expressed barely detectable levels of neurofilament in this system. However, T cells activated through stimulation with PMA expressed significant levels of phosphorylated neurofilament which was detected using the RT97 antibody. Anti-CD3 similarly induced a significant increase in detectable phosphorylated NF 200 as detected using monoclonal antibody RT97 (Fig. 3d). In these experiments, we also detected increased levels of NF 200 utilizing the monoclonal antibody 2F11 which is not phosphorylation dependent (Fig. 3c). However, the detection appeared somewhat fainter than RT97. This may relate to the manner in which the epitope recognized by 2F11 is presented on immunoblots as this antibody was superior to RT97 for staining cells *in situ*.

We examined the pattern of expression of neurofilament in HuT 78 cells following stimulation with anti-LFA-1 as previously described.[4] Briefly, cytoskeletal studies were performed by pre-coating Labtek chamber slides (Nunc, Napierville, IL) with goat anti-mouse immunoglobulin overnight at 4°. Chambers were then coated with antibodies to LFA-1α, or CD3, washed, HuT 78/2 cells added (10^6 cells/ml) and cultured at 37° for 4 hr in 5% CO_2. At 4 hr the chambers were washed well in phosphate-buffered saline (PBS), the cells were then fixed in acetone and membranes permeabilized using saponin 0·1%. Cytoskeletal staining was then performed using anti-neurofilament 2F11 (Monosan) and RT97 and an alkaline phosphatase-anti alkaline phosphatase (AP-AAP) technique (Dakopatts, Glostrup, Denmark). Briefly, HuT 78 T cells were found to contain the majority of the neurofilament condensed into a perinuclear spheroid structure (Fig. 4c). A quantity of neurofilament was found to lie in a fan-like structure expanding from this condensed structure in some cells. The intracellular organization of neurofilament was altered by stimulation of the cells with anti-LFA-1. Hence T cells stimulated with anti-LFA-1 demonstrated a reorganization of the cytoskeleton with the unpacking of the perinuclear core and the development of dendritic processes containing neurofilament (Fig. 4d).

In this study, we have demonstrated that both circulating T cells and a T-cell lymphoma line expressed significant quantities of neurofilament of molecular weight 200,000. Neurofilament expression has been thought in the past to be exclusively associated with cells originating in the embryonic CNS. While neurofilament has recently been described as a cytoskeletal component in a thymoma derived from thymic epithelial cells,[5] it has not previously been reported in T-cell tumours. It is notable however, that the fidelity of intermediate filament expression for embryonal origin of cells is not confirmed for tumour cell lines.[7] Hence, squamous carcinoma cells have been shown to express neurofilament in culture, while renal carcinoma cells are known to express both keratin and vimentin.[8] We have previously demonstrated vimentin expression in HuT 78 cells[4] which appeared in a similar intracellular distribution to neurofilament. In transfection studies where NF was transfected into Cos cells, the intracellular distribution of NF suggested that vimentin and NF could form filamentous heteropolymers.[9] Furthermore NF in the resting state accumulated in a perinuclear location coinciding with the centrosome of the cell. It has thus been suggested that this location is the organizing centre for both the microtubule and the intermediate filament cytoskeleton.[10]

The demonstration of activation of neurofilament by stimulation through a specific surface receptor for T-cell activation and its regulation by a leucocyte integrin suggests that this is an integral part of T-cell activation. Furthermore, the similarity of the effects mediated through the T-cell receptor and PMA suggest that this effect is mediated through the action of protein

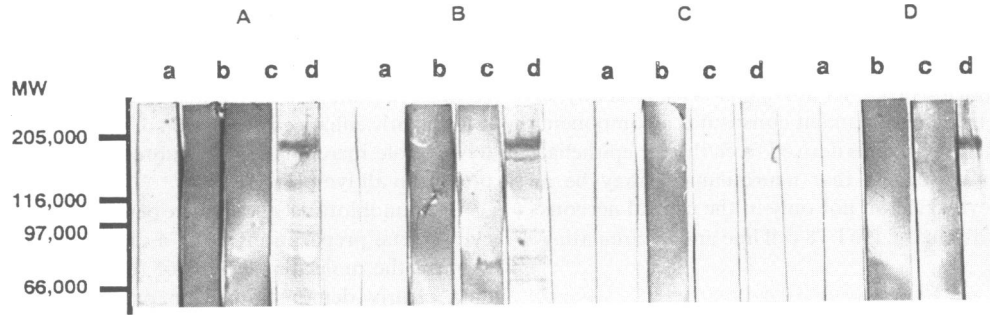

Figure 2. Immunoblot analysis of neurofilament expression in neuroblastoma cells (A), HuT 78/2 cells (B), resting T cells (C) and PMA-activated T cells (D). Antibodies used: (a) no primary antibody; (b) anti-murine IE; (c) anti-LFA-1; (d) antibody RT97 (directed against phosphorylated neurofilament). This blot is representative of three similar experiments.

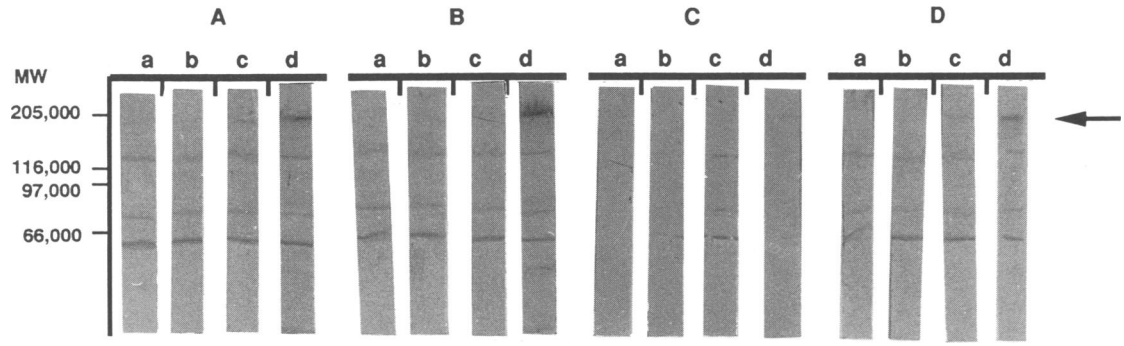

Figure 3. Immunoblot analysis of neurofilament expression in neuroblastoma cells (A), HuT 78/2 cells (B), resting T cells (C) and OKT3-activated T cells (D). Antibodies used: (a) no antibody; (b) anti-murine IE; (c) anti-neurofilament 2F11; (d) anti-phosphoneurofilament RT97. The arrow indicates the neurofilament 200,000 MW band. Remaining bands are non-specific. This blot is representative of three similar experiments.

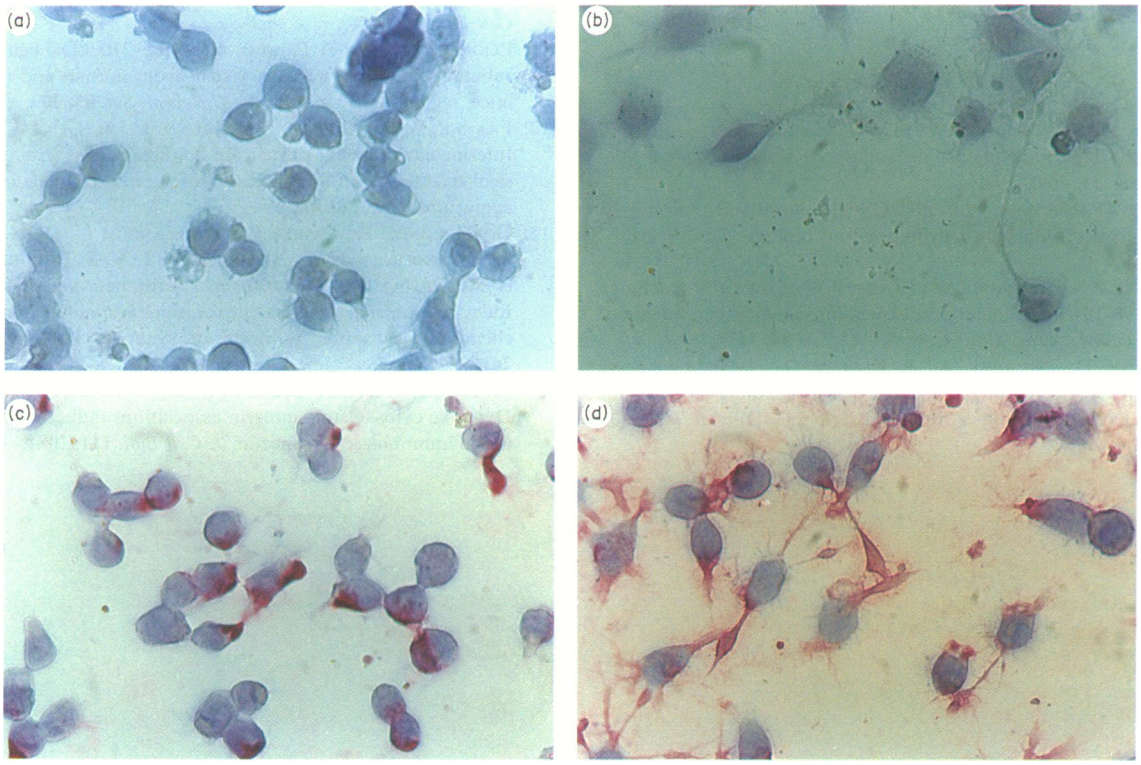

Figure 4. Reorganization of neurofilament in HuT 78/2 cells following stimulation with anti-LFA-1. HuT 78 cells were incubated on chamber slides precoated with control antibody or anti-LFA-1. After 4 hr, membranes were permeabilized and cytoskeletal staining performed using monoclonal antibodies 2F11 and RT97 to neurofilament. (a) Cells preincubated in the absence of anti-LFA-1. (b) Cells stimulated by activation with anti-LFA-1. (a,b) Stained with control antibody only. (c,d) Unstimulated and anti-LFA-1-stimulated HuT 78/2 cells stained with anti-neurofilament antibody. Red staining indicates neurofilament detection. Results are representative of three similar experiments. Cells were incubated for 4 hr in the presence of anti-LFA-1 antibody prior to staining for neurofilament as described in ref. 4. Control antibodies including antibody to HLA class I (human), class II and anti-CD3 (OKT3) did not induce dendritic processes in this cell line.

kinase C. However, neurofilament synthesis in the CNS could be increased by interferon[11] and it is conceivable that the increased synthesis detected in T cells is secondary to production of interferon or other cytokines.

The functional significance of neurofilament expression and reorganization in human T cells is potentially interesting. It has been speculated that intermediate filaments function to form a bridge between the plasma membrane and the nuclear membrane.[12] Neurofilaments certainly have a structural role in the maintenance and synthesis of cell structure in neuronal cells. More recently relationships between synapsin and with fibronectin have suggested a role for NF in signalling[13] through second messengers. Secondly, the finding of a dynamic interaction between intermediate filaments and matrix molecule recep-

tors[14] suggests an involvement with the integrin system of cell surface receptors. This may be important in increasing the surface area of the cell, this permitting a T cell to access and signal (or receive signals from) cells remote from its direct site and localization. This function would be of particular importance in mucosal sites such as skin from which the HuT 78 cell line was derived. Alternatively, development of dendritic processes containing neurofilament in response to anti-LFA-1 stimulation suggest the possibility that signalling through integrin receptors involving changes in the intermediate filament cytoskeleton might play a role in lymphocyte migration.

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