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Tumour-associated upregulation of the IL-4 receptor complex

I. Mat[1], M. Larche[1], D. Melcher[2] & M.A. Ritter[1]

[1]*Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN and* [2]*Department of Histopathology, Royal Sussex County Hospital, Brighton, UK.*

Summary In a previous study we have shown that monoclonal antibody MR6, which we believe recognizes a component of the human IL-4 receptor complex, binds to a wide variety of epithelial tumours. We have now used this reagent to carry out more detailed analysis of tumours of the breast. Our immunohistochemical data indicate that approximately 30% of these tumours show elevated expression of the molecule to which MR6 binds. In addition, three samples of lymphoma were all MR6-positive. Normal breast tissue from the same patients was either negative or weakly positive. Immunoprecipitation and Western blotting analysis with MR6 show that the molecule expressed on tumour cells is indistinguishable from that on normal tissues. It has an apparent molecular weight of 200 kD, but is highly sensitive to proteolysis yielding a molecule of 145 kD. These data raise the possibility that upregulation of the IL-4 receptor complex may be involved in tumourigenesis. In addition, since only a third of tumours are MR6-positive, the antibody may have potential in differential diagnosis.

The monoclonal antibody MR6 was raised to epithelial cells of the thymic cortex as part of a study designed to analyse the inductive and proliferative signals provided by the thymic microenvironment for developing intrathymic T lymphocytes (De Maagd *et al.*, 1985). The molecule recognized by MR6 (MR6-Ag) was subsequently found to be present at low levels on the surface of T and B lymphocytes, macrophages and dendritic cells (Larche *et al.*, 1988*b*; von Gaudecker *et al.*, 1989). However, of all normal tissues screened, only thymic epithelium showed strong expression. Subsequently, MR6 was found to block IL-4 induced immune responses, suggesting that the molecule to which the antibody binds may form part of the IL-4 receptor (IL-4R) complex (Larche *et al.*, 1988*a*).

In a recent study we have found that many different tumours of epithelial origin are strongly MR6-positive, whereas the equivalent normal tissue was either MR6-negative or weakly positive (Al Jabaari *et al.*, 1989). These findings, together with preliminary imaging data, indicate that elevated expression of the IL-4 receptor complex may be involved in the process of tumourigenesis and that the monoclonal antibody MR6 may be useful both as an *in vitro* diagnostic reagent and for *in vivo* imaging and therapy of epithelial tumours.

We have now followed this initial broad survey with a more detailed analysis of tumours of the breast. Our data indicate that approximately 30% of these tumours have elevated expression of the MR6-Ag/IL-4R complex, and that the polypeptide detected on tumour cells is indistinguishable from that on their normal counterparts.

Materials and methods

Tissue samples

The following snap frozen biopsy samples from patients attending the Royal Sussex County Hospital were analysed: carcinoma of the breast, non-malignant breast proliferation, normal breast tissue and three cases of lymphoma.

Immunohistochemical analysis

Frozen sections (5 μm) were analysed by indirect immunofluorescence and immunoperoxidase using mouse monoclonal antibody MR6 or an isotype-matched (IgG_1) negative control. MR6-positive tumours were further analysed with monoclonal antibody MR6 and a rabbit polyclonal anti-keratin antibody (Dakopatts, Copenhagen, Denmark) by two-colour immunofluorescence. No dual staining was observed when either MR6 or anti-keratin first layer antibodies were omitted, demonstrating the specificity of the two-colour immunofluorescence system used. Details of these methods have been published elsewhere (Al Jabaari *et al.*, 1989).

Immunoprecipitation and Western blotting

Immunoprecipitation Cells were surface iodinated using lactoperoxidase, hydrogen peroxide and 125-iodine. After washing, cells were lysed in 10 mM Tris buffer (pH 7.4) containing 1 mM $MgCl_2$, 0.5% NP40, 10 μg ml^{-1} aprotinin and 1 mM phenyl methyl sulphonyl fluoride (PMSF). Mouse serum and protein A beads (Pharmacia, Uppsala, Sweden) were added to absorb all non-specific binding. For specific precipitation, MR6 was added to the lysate at a final concentration of 50 μg ml^{-1}. After 3 h an excess of protein A beads was added and incubated together for 3 h. The beads and bound antigen/antibody complexes were washed five times in washing buffer 50 mM Tris/HCl (pH 8.0) containing 0.5 M NaCl, 10 mM EDTA, 0.1% SDS, 0.5% NP40, followed by boiling for 3 min in 2 × Laemlli sample buffer. Precipitated proteins were then separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (7.5% SDS-PAGE). Proteins were visualized by autoradiography using Ortho G X-ray film (Kodak, UK).

Western blotting This technique was carried out as previously described with the exception of the addition of 1 mM PMSF (Larche *et al.*, 1988*b*; Al Jabaari *et al.*, 1989).

Results

Immunohistochemical analysis

Immunoperoxidase and immunofluorescence labelling was scored by eye for the approximate percentage of positive cells in each tissue sample. Normal breast tissue contained 5–20% MR6-positive cells; these were predominantly macrophages and dendritic cells, as has been described previously (De Maagd *et al.*, 1985). Tumours fell into two categories: those that contained 0–20% MR6-positive cells, mainly macrophages, and those where the percentage of MR6-positive cells was >50%. Dual immunofluorescent analysis of the latter group showed that >80% of the MR6-positive cells were also keratin-positive epithelial cells. These epithelial tumour cells were much more intensely labelled than normal breast epithelium, which was either weakly positive or completely negative. Not all tumours were MR6-positive. The frequency

Correspondence: M.A. Ritter.

of MR6-positive samples was as follows: 5/20 breast carcinoma, 3/3 non-malignant proliferation of breast tissue, 3/3 lymphoma. The data are summarized in Figures 1 and 2.

Biochemical analysis

Lysates of normal thymus (chosen because it contains normal epithelium that expresses high levels of the MR6 antigen), malignant T (CCRF-CEM) and B (Daudi) lymphocyte cell lines and carcinoma tissue were analysed in parallel by immunoprecipitation and Western blotting. Both techniques yield a single band of 200 kD when PMSF is included in the lysate buffer. In the absence of this protease inhibitor, the 200 kD band is either partially or totally cleaved to 145 kD. The apparent molecular weight of these bands is the same when the SDS-PAGE is run under non-reducing and reducing conditions. The data are illustrated in Figure 3.

Discussion

We have previously shown that many different carcinomas exhibit unusually strong labelling with monoclonal antibody MR6, suggesting an elevated expression of the IL-4R complex (Al Jabaari *et al.*, 1989). However, in this preliminary survey the number of tumours of any given type was too small to determine the frequency and diagnostic significance of such observations. We have therefore studied single groups of tumours in greater depth and now report our findings for 23 tumours of the breast. Our data show that approximately a third of epithelial tumours label strongly with monoclonal antibody MR6, in contrast to normal breast epithelium which is either MR6-negative or weakly positive. Similarly, three samples of lymphoma were also strongly positive, although normal lymphocytes have been shown to bear only low levels of MR6-Ag (Larche *et al.*, 1988*a*). Immunoelectron microscopy has shown that the MR6-Ag is located on the cell surface of normal lymphocytes and epithelial cells (von Gaudecker *et al.*, 1989). However, the intensity of labelling of some carcinomas (Figure 2) suggests that there may also be a pool of intracellular MR6-Ag. Further studies at the electron microscope level are required to clarify this point.

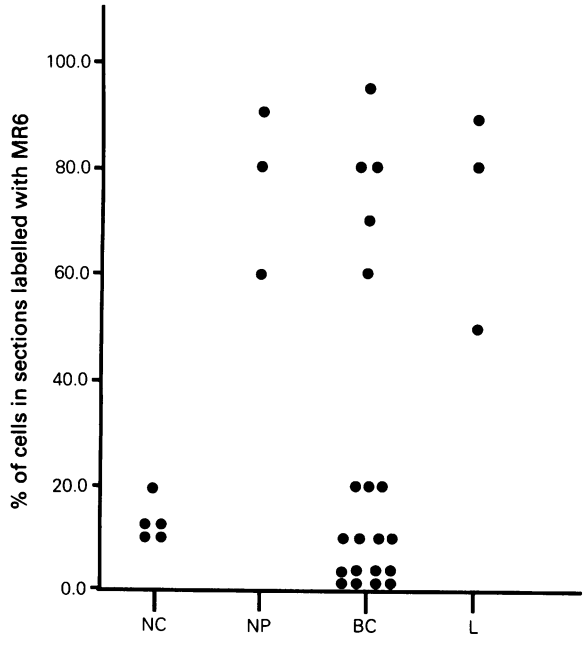

Figure 1 Analysis of IL-4R complex expression in breast tumours (BC), non-malignant proliferation of breast (NP), normal breast tissue (NC) and lymphoma (L). Frozen tissue sections were labelled with MR6 monoclonal antibody by indirect immunoperoxidase and scored for the percentage of MR6-positive cells. Each dot represents tissue from a single patient.

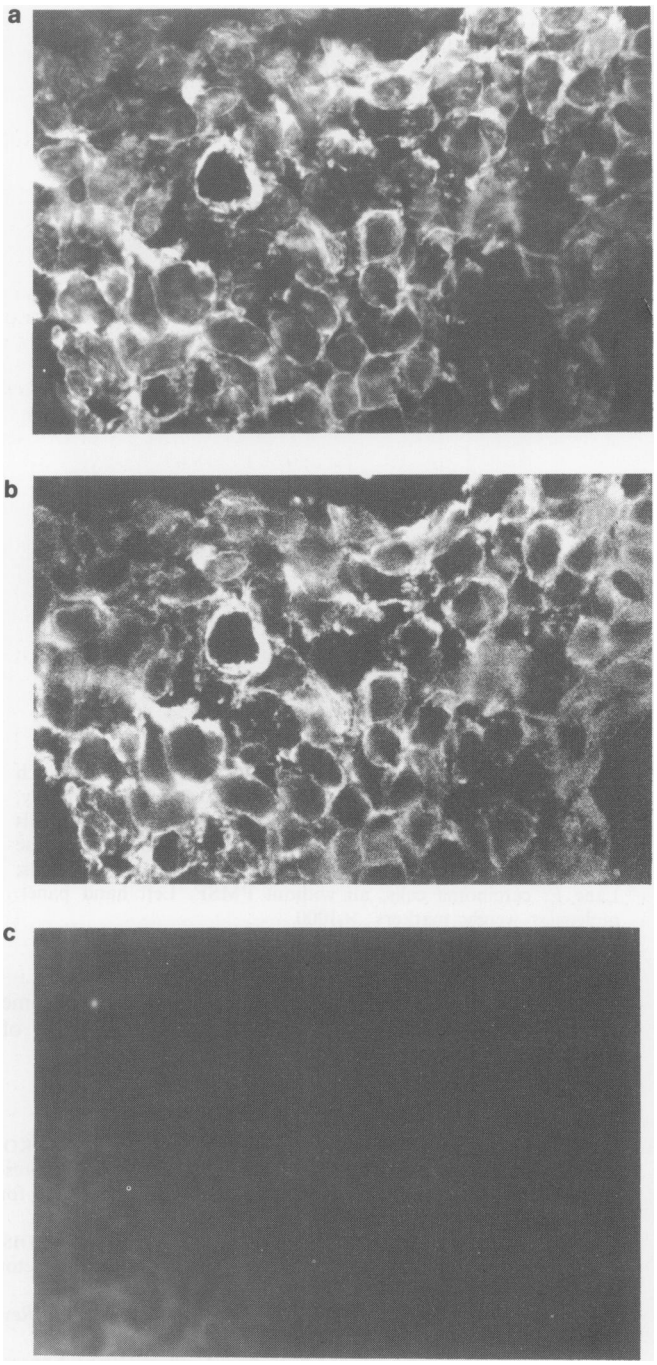

Figure 2 Elevated expression of IL-4R complex on breast tumours. Frozen sections were labelled in dual immunofluorescence with monoclonal antibody MR6 (fluorescein) and polyclonal rabbit anti-keratin (rhodamine) in dual immunofluorescence. The figure shows a single section labelled with **a** anti-keratin and **b** MR6: the majority of epithelial cells in this case of breast carcinoma are MR6-positive. **c** Isotype matched control gives no staining.

IL-4 is known to act as a growth and/or differentiation factor for T and B lymphocytes, macrophages and mast cells (Howard *et al.*, 1982; Vitetta *et al.*, 1985; Coffman *et al.*, 1986; Lee *et al.*, 1986; Mossman *et al.*, 1986; Hu-Li *et al.*, 1987), following binding to its receptor that is present at low density on the surface of these cells. However, although we have demonstrated high levels of MR6-Ag/IL-4R on the surface of epithelial cells in the thymic cortex, it is not clear what the functional effect of IL-4 on these non-mitotic cells might be (De Maagd *et al.*, 1985; Larche *et al.*, 1988*a*; von Gaudecker *et al.*, 1989). Epithelia in other organs bear few or no IL-4R, although interestingly those that are weakly MR6-positive are found in germinative layers, suggesting that IL-4 may act as growth factor for some epithelial cells.

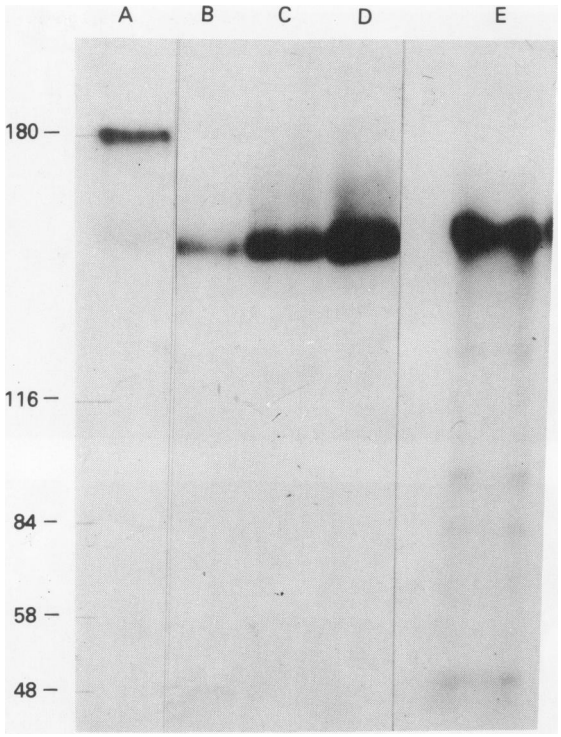

Figure 3 Immunoprecipitation analysis of the molecule to which monoclonal antibody MR6 binds, showing protease sensitivity. Lanes A and D: Daudi (EBV transformed B lymphocytes) cells with (A) or without (D) 1 mM PMSF to inhibit proteolysis; Lane B: normal thymus tissue; Lane C: CCRF-CEM T leukaemic cells; Lane E: carcinoma cells, all without PMSF. Left hand panel: molecular weight markers × 1000.

Our finding that MR6-Ag/IL-4R are upregulated on some tumours raises the possibility that elevated expression of receptor molecules may play a key role in hyperproliferation and ultimately in the process of tumourigenesis. IL-4R might therefore be acting as an oncogene product in a similar way to that suggested for other growth factor receptors (Land *et al.*, 1983; Bishop, 1983; Hudziac *et al.*, 1987). The fact that MR6-Ag expression is elevated on tumours and that our biochemical analysis using monoclonal antibody MR6 can detect no difference between the molecule on normal and tumour cells suggests that gene amplification rather than truncation is likely to be responsible for the tumourigenic effects. Alternatively, a point mutation in the MR6 gene could be involved.

Other growth factor products of oncogenes are known to be elevated in epithelial tumours. For example, 80% of lung squamous cell carcinoma show elevated epidermal growth factor receptor (EGFR) expression, and 20% of breast carcinoma have amplified copies of the c-*erb*-B gene (Cerny *et al.*, 1986; Berger *et al.*, 1987; Slamon *et al.*, 1987). Whether amplification of these oncogene products is mutually exclusive or whether they jointly contribute to the multistage process of oncogenesis is not known. We are currently analysing the breast tumour samples in our series with antibodies to these and other tumour-associated molecules to elucidate this question.

Our data concerning the elevation of MR6-Ag/IL-4R on breast tumours may be of considerable use in clinical medicine. Oncogene amplification has recently been shown to correlate with disease prognosis in carcinoma of the breast, lung and bladder (Berger *et al.*, 1987; Slamon *et al.*, 1987; Harris *et al.*, 1988). MR6-Ag expression may therefore be similarly useful in prognostic evaluation. In addition, since any given oncogene product is amplified in only a minority of such tumours, this raises the possibility that different subtypes of a tumour may be characterized by upregulation of different oncogene products. The MR6 monoclonal antibody may therefore have considerable potential in differential diagnosis, although further detailed studies correlating MR6-Ag/IL-4R expression with disease diagnosis are required before such potential could be realized.

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