ability to cleave this mutant antibody not only at 227–228 but also at or close to the Pro-235–Thr-236 peptide bond, which is the specific cleavage site in wild-type IgA1 for type 2 IgA1 proteases of N. meningitidis and H. influenzae.

References

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Targeted cytokine delivery to neuroblastoma
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Abstract
The aim of this study was to construct a fusion protein from the cytokine granulocyte/macrophage colony-stimulating factor (GM-CSF) and a single-chain Fv fragment (scFv D29) and to investigate its potential to activate cells of the immune system against neuroblastoma cells expressing neural cell adhesion molecule (NCAM). Mammalian cell expression of the scFv D29-GM-CSF fusion protein was compared using a number of vectors, including retroviral and adenoviral vectors. The resultant fusion protein, expressed by HeLa cells, was found by ELISA to bind immobilized recombinant NCAM. Moreover, FACS analysis confirmed binding to the human neuroblastoma cell line SKNBE and a murine neuroblastoma cell line engineered to express the glycosylphosphatidylinositol form of human NCAM (N2A-rKNIE). The fusion protein was also found to stimulate the proliferation of the FDC-P1 haemopoietic cell line, which is dependent on GM-CSF (or interleukin 3) for continued growth. In vitro clonogenic assays indicated that scFv-GM-CSF could selectively induce growth inhibition of SKNBE cells by murine lymphoid cells.

Introduction
Neuroblastoma is the most common solid malignant tumour in children after brain tumours, accounting for 40% of solid tumours presenting in the first 4 years of life. Neuroblastomas are derived from the neural crest, and have heterogeneous biological, genetic and morphological characteristics. The majority of patients that are diagnosed with neuroblastoma present at stage 4 (of the International Neuroblastoma Staging System), with metastatic disease that may initially respond to therapy, but which has a high probability of recurring. These patients have a low overall survival rate (less than 30%), despite undergoing surgery and moderately aggressive forms of cytotoxic therapy [1].

Recent advances in the fields of antibody engineering and production have led to the design and use of a number of monoclonal antibodies for cancer therapy; for example, Herceptin, Mylotarg, Rituximab and Infliximal. In addition, systemic treatment with cytokines such as interleukin 2 and granulocyte/macrophage colony-stimulating factor (GM-CSF) has been shown to increase the immunogenicity of tumours. However, frequent side effects associated with toxicity limit the effectiveness of cytokine therapy in vivo. The conjugation of cytokines with monoclonal antibodies has been demonstrated to concentrate cytokines within the tumour micro-environment, thereby inducing tumour-specific

Key words: granulocyte/macrophage colony-stimulating factor (GM-CSF), scFv.
Abbreviations used: NCAM, neural cell adhesion molecule; GM-CSF, granulocyte/macrophage colony-stimulating factor; scFv, single-chain Fv.
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immune responses and reducing overall systemic toxicity [2,3]. In comparison with intact antibodies, single-chain Fc (scFv) molecules show increased tumour penetration, possibly as a consequence of their reduced size (27 kDa as opposed to 150 kDa for an IgG molecule) [4].

In this study, the gene for a humanized scFv (termed D29), derived from the anti-neuroblastoma cell adhesion molecule (NCAM) monoclonal antibody Eric-1, was linked to the murine GM-CSF gene and spliced into a number of vectors for expression of the scFv D29-GM-CSF fusion protein in mammalian cell lines. The resultant fusion protein was characterized and its potential to selectively induce growth inhibition of human neuroblastoma SKNBE cells by murine lymphoid cells in vitro investigated.

**Results**

Mammalian cell expression of the scFv D29-GM-CSF fusion protein was achieved using a number of vectors, including a retroviral vector (the KAT...
vector system [5]) and an adeno viral vector (the Cre8/pADLOX adenoviral system [6]). FACS analysis confirmed specific binding of the D29-GM-CSF fusion protein to the human neuroblastoma cell line SKNBE and a murine neuroblastoma cell line engineered to express the glycosylphosphatidylinositol form of human NCAM (N2A-rKNIE) as opposed to binding by an irrelevant scFv-GM-CSF fusion protein (known as B1.8-GM-CSF, against the 3-iodo-4-hydroxy-5-nitrophenacetyl antigen; Figure 1) [8].

Both the D29-GM-CSF and B1.8-GM-CSF fusion proteins were found to stimulate the proliferation of the FDC-P1 haemopoietic cell line, which is dependent on GM-CSF (or interleukin 3) for continued growth. Furthermore, in vitro clonogenic assays using murine lymphoid cells isolated from spleen indicated that D29-GM-CSF could selectively induce growth inhibition of SKNBE cells (Figure 2). The results show that the addition of murine effector cells to the human neuroblastoma cell line induced a growth-inhibition effect (Figure 2, bar no. 4), which was not unexpected. The effect was not significantly enhanced by the addition of an irrelevant scFv-GM-CSF fusion protein. The addition of the anti-NCAM scFv-GM-CSF fusion protein did induce a small additional inhibition of growth compared with effectors alone ($P = 0.00658$), or effectors and irrelevant fusion protein ($P = 0.00184$).

**Conclusions**
The construction of an scFv-GM-CSF fusion protein targeting neuroblastoma cells expressing NCAM and its potential to inhibit the growth of a human neuroblastoma cell line was investigated. Retroviral and adeno viral vectors containing the gene for the fusion protein were constructed in order to overcome the complexity of large-scale protein production and purification. Adenoviruses in particular have been used in a number of animal models for in situ production of proteins [7,8]. In vitro clonogenic assays indicated that the anti-NCAM scFv-GM-CSF could selectively induce growth inhibition of human neuroblastoma cells by murine lymphoid cells.

**References**