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CONSTRUCTION AND CHARACTERISATION OF A FUNCTIONAL CD19 SPECIFIC SINGLE CHAIN Fv FRAGMENT FOR IMMUNOTHERAPY OF B LINEAGE LEUKAEMIA AND LYMPHOMA

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Abstract—The B cell specific antigen CD19 is a target for the immunotherapy of B lineage leukaemias and lymphomas. We have engineered a single chain Fv (scFv) fragment from the mouse hybridoma cell line FMC63 which produces monoclonal antibody specific for CD19. The genes encoding the FMC63 heavy and light chain variable regions were amplified from cDNA and a scFv was constructed by splice overlap extension PCR. Analysis of staining of lymphoblastoid cell lines, peripheral blood lymphocytes and tonsil sections demonstrated that the monovalent scFv fragment has the same cellular specificity as the parent hybridoma antibody. Kinetic studies with radiolabelled material showed that the scFv binds target cells with a K_a of 2.3×10^{-9}, compared with 4.2×10^{-9} for the parent antibody. This CD19 scFv will be used in experimental models to test its therapeutic efficacy and immunogenicity, with a view to application in the diagnosis and treatment of human B cell cancers.

Key words: scFv, CD19, antibody therapy, leukaemia, lymphoma.

INTRODUCTION

Antibody directed imaging and immunotherapy of tumours relies on targeting tumour-associated antigens. CD19 is expressed on most B lineage malignancies, including acute lymphoblastic leukaemia, chronic lymphocytic leukaemia and non-Hodgkin's lymphoma. Because CD19 is absent from bone marrow progenitor cells it is a potential target for immunotherapy of these malignancies (Uckun *et al.*, 1988). Antibodies against CD19 inhibit the growth of tumour cells (Ghetie *et al.*, 1994). CD19 is not readily shed from cells (Uckun *et al.*, 1988) and is internalised with bound antibody, allowing delivery of anti-CD19-linked toxins (Uckun *et al.*, 1988). Animal models have indicated the potential value of antibodies to CD19 (Jansen *et al.*, 1992; Pietersz *et al.*, 1995). Antibody alone (Hekman *et al.*, 1991), with IL-2 (Vlasveld *et al.*, 1995), or conjugated to toxin (Grossbard *et al.*, 1993; Stone *et al.*, 1996) have been used in clinical trials for therapy of leukaemia and lymphoma and CD19 scFv have been described (Bejcek *et al.*, 1995).

Some of the limitations of therapeutic monoclonal antibodies can be overcome by engineering smaller and more effective antibody fragments (Winter *et al.*, 1994). scFv are single gene fusions of the antibody heavy and light chain variable regions joined by a peptide linker. Because they are smaller than whole antibodies, scFv show improved penetration into poorly vascularised tumours (Yokota *et al.*, 1992) and in clinical trials have shown negligible immunogenicity (Begent *et al.*, 1996). Functional moieties such as toxins, enzymes, or sites for binding drugs or radioisotopes can be incorporated (Ghetie and Vitetta 1994; Pietersz *et al.*, 1992). Engineered antibody fragments can be produced on a large scale in bacterial or mammalian expression systems (Pack *et al.*, 1993; Bebbington, 1995).

We describe the production and characterisation of a CD19 scFv, CHRI-19Fv1. Staining of lymphoblastoid cell lines, peripheral blood lymphocytes and tonsil sections indicates that CHRI-19Fv1 has the same cellular specificity as the parent antibody and has retained a high level of binding albeit with a 2-fold reduction in affinity

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