Functional scFv Antibody Sequences against the Organophosphorus Pesticide Chlorpyrifos

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Two functional single-chain Fv (scFv) antibodies that recognize specifically the widely used organophosphorus pesticide chlorpyrifos-ethyl were derived from two murine hybridoma cell lines. It is shown that the functional scFvs could be isolated without any rounds of selection, with a success rate dependent on the efficiency of amplification of the functional light chain gene family by the specific primers. Besides four new functional immunoglobulin variable gene sequences, the isolation of a new pseudogene is reported.

Keywords: *Variable region; antibody engineering; immunoassay; recombinant antibody; antibody pseudogene*

Recombinant antibodies offer several potential advantages over polyclonal and monoclonal antibodies, including the speed of antibody generation, the ability to generate novel and rare functionality, and the possibility of altering affinity and specificity (Huston et al., 1993). Spleen cells, hybridomas, and synthetic libraries have been used as sources of recombinant antibody fragments (Hudson, 1998), although the generation of functional fragments from all three sources has been problematic. For hybridomas, the presence of aberrant mRNAs has been extensively reported (Carroll et al., 1988; Duan and Pomerantz, 1994; Krebber et al., 1997; Nicholls et al., 1993; Ostermeier and Michel, 1996). The levels of aberrant mRNAs can greatly exceed the levels of normal antibody transcripts (Ostermeier and Michel, 1996) and hinder the successful generation of single-chain fragments of antibody variable regions (scFvs). As an example, hybridoma cell lines derived from the widely used P3-X63-Ag8.653 myeloma cell line are reported to bear high levels of rearranged pseudogenes (Carroll et al., 1988). Since the problem was recognized, several procedures have been proposed to eliminate the nonfunctional pseudogenes, including RNase H digestion (Ostermeier and Michel, 1996), ribozyme cleavage (Duan and Pomerantz, 1994), in vitro translation (Nicholls et al., 1993), and phage display selection (Krebber et al., 1997). A critical discussion on the advantages of the different methods is given by Krebber et al. (1997).

Here we describe the isolation of two functional scFvs that recognize specifically chlorpyrifos-ethyl [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphothioate], a broad spectrum organophosphate insecticide used widely in agricultural and domestic infestations (Worthing and Hance, 1991). The pesticide can occur as a contaminant in food and in the environment (Manclus et al., 1996), and immunoassays with polyclonal and monoclonal antibodies have been described with properties that are superior to the more traditional but time-consuming chromatographic procedures (Hill et al., 1994; Lawruk et al., 1996; Manclús et al., 1996). We show that the functional scFvs were isolated without any rounds of selection, with a success rate dependent on the efficiency of amplification of the functional light chain gene family by the specific primers. Besides four new functional immunoglobulin variable gene sequences, we also report the isolation of a new pseudogene.

The murine hybridoma cell lines IFRN 1301 and IFRN 1302 were obtained from two immunized animals and distinct cell fusion procedures separated by a time interval of six months. Recombinant scFv antibodies were constructed by assembling the amplified heavy chain variable domain (VH) and light chain variable domain (VL) cDNA with an artificial linker of 20 amino acids, essentially as described by Krebber et al. (1997), using Master Amp 2xPCR premix buffer B (Epicentre Technologies) for the amplification and premix buffer J for the SOE-PCR. The scFv constructs were cloned in the pAK100 vector (Krebber et al., 1997) and electroporated into *Escherichia coli* XL1-Blue (Stratagene) according to standard procedures. Screening was performed by expressing the individual transformed colonies (1 mL) in the presence of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 4 h at 25 °C with shaking. After centrifugation, the periplasmic extracts were produced by resuspending the *E. coli* pellets in 300 μL of ice-cold TES (0.2 M Tris-HCl, pH 8.0/0.5 M EDTA/0.5 M sucrose) buffer as previously described (Harrison et al., 1996). In-frame scFv expression was monitored by the detection by enzyme-linked immunosorbent assay (ELISA) of the c-Myc peptide fused at the C-terminal end (Cravchick and Matus, 1993). Functional scFvs were detected in an indirect ELISA as previously described for parathion (Garret et al., 1997), using 10 μg/mL of chlorpyriphos-ethyl dextran (Manclús et al., 1996) for microtitration plate coating, chlorpyriphos-ethyl as competitor, and the 9E10 mAb for c-Myc detection. Sequencing was performed on an automated DNA sequencer using the Big Dye terminator cycle sequencing kit (Applied Biosystems).

Functional scFvs were found without any prior selection (Table 1). A significant difference in numbers of functional scFvs was observed between the two cell

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Table 1. Variable Chain Content of the scFvs Produced from Hybridoma Cell Lines Secreting Monoclonal Antibodies IFRN 1301 and IFRN 1302 Screened for the Presence of c-Myc and for Binding to Chlorpyrifos in the ELISA

	IFRN 1301[a]					IFRN 1302[b]				
ELISA[c]	VH	%	VL	%	total %	VH	%	VL	%	total %
c-Myc +/Chl +	heavy 2	100	light c	100	18	heavy 1	100	light d	100	1
c-Myc+ /Chl −	heavy 1	100	light c light a[d]	67 33	42					0
c-Myc −	heavy 1 heavy 2	75 25	light a light b	75 25	40	heavy 1 heavy 2	80 20	light a light b	60 40	99

[a] Ninety-six colonies were screened. [b] Seventy-two colonies were screened. [c] c-Myc peptide detection and chlorpyrifos-ethyl binding. [d] Mis-sense mutation S105*.

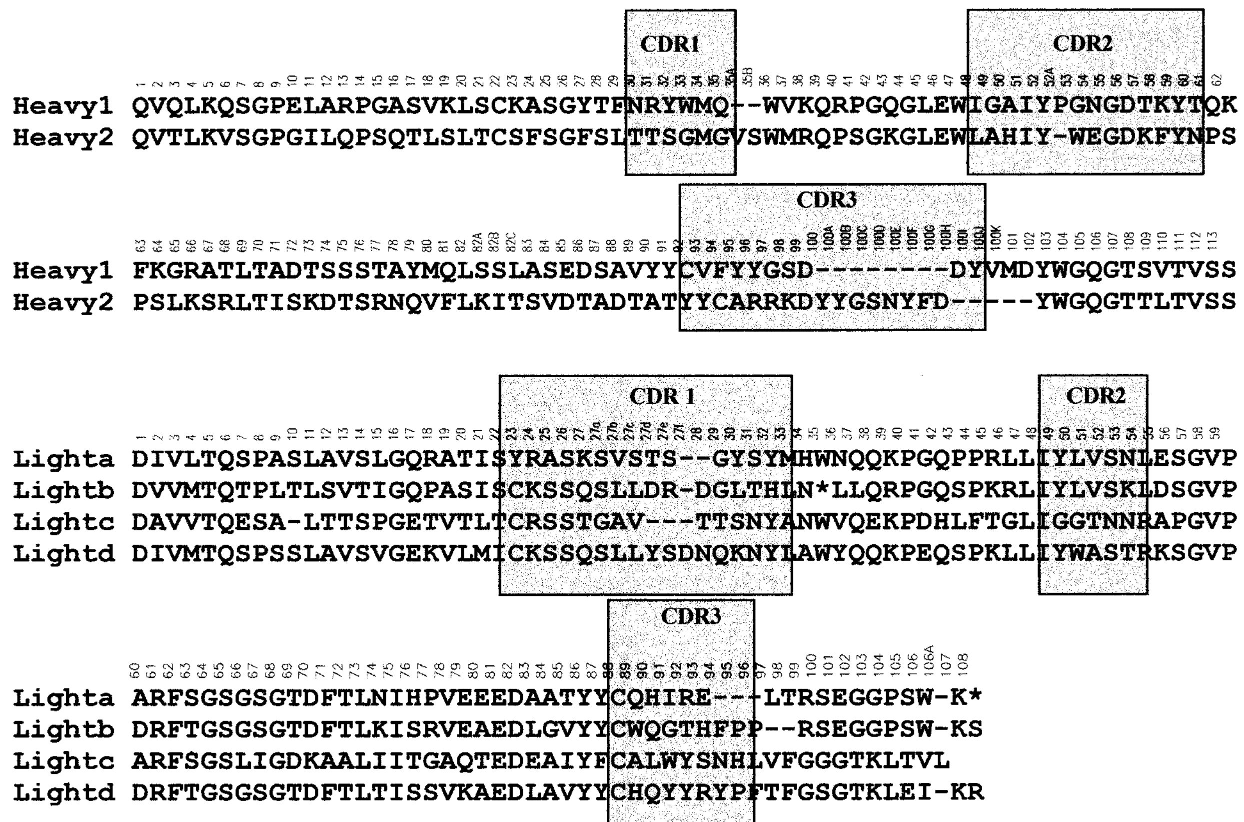

Figure 1. Amino acid sequence alignment of antibody variable domains expressed by hybridoma cell lines IFRN 1301 and IFRN 1302. Residue numbers are aligned over the Kabat database (http://immuno.bme.nwu.edu/scripts/websearch.tcl). The seven amino acids at each end are encoded by PCR primer sequences. Heavy 1, mAb IFRN 1302 functional VH transcript (AF132308); heavy 2, mAb IFRN 1301 functional VH transcript (AF132309); light a, nonfunctional VL-κ pseudogene transcript found in P3-X63-Ag8.653 myeloma cell line (Carroll et al., 1988) (M35669); light b, nonfunctional VL-κ pseudogene transcript (AF106674); light c, functional VL-λ transcript found in mAb IFRN 1301, identical to germline VL-λ sequence (Weiss and Wu, 1987) except for L96Y (AF132309); light d, functional VL-κ transcript found in mAb IFRN 1302 (AF132308). Genbank accession numbers are given in parentheses.

lines, suggesting a bias toward the mAb IFRN 1301 gene family during Polymerase Chain Reaction (PCR) amplification. To understand the origin of these results, we sequenced nonfunctional scFvs from both cell lines. The functionality of the scFvs was found to be linked to the specific constructs: neither the chimeras from the two functional scFvs (heavy 2 × light d, shuffling experiment not shown) nor the combination of any of the heavy chains with light chains from other cell lines (data not shown) was able to bind the pesticide.

As expected, the nonfunctional pairs contained a significant level of the light chain a pseudogene derived from the myeloma cell line P3-X63-Ag8.653 (Figure 1 and Table 1). In a few cases, the primer used mutated artificially the stop codon to a serine in the light chain a sequence producing full-length constructs. In both cell lines we also found a second pseudogene, light chain b, that has not been described before (Figure 1). scFv IFRN 1301 possessed a heavy chain (heavy 2) that was classified according to the amino acid sequence as a member of the family XIX subgroup IB and a λ light chain (light c) from the family I subgroup II (Kabat, 1991), whereas IFRN 1302 possessed a heavy chain (heavy 1) that belonged to the family VIII subgroup IIA and possessed a κ light chain (light d) from the family II subgroup I. The presence of the same two heavy chains in the scFvs from both cell lines was unexpected. At present, possible cross-contamination cannot be excluded. However, the occurrence of the two heavy chains in the same proportions in both cell lines and the absence of contaminant functional light chains in either group suggest that the heavy chains could have been present initially in both cell lines.

As shown in Table 1, the κ light chain d is the limiting factor in the generation of functional scFvs from mAb IFRN 1302. The successful amplification of light chain c from the less variable λ gene family explains the higher levels of functional scFvs found in mAb IFRN 1301. These results taken together emphasize the importance of the use of an extended primer mix as discussed by Krebber et al. (1997).

ABBREVIATIONS USED

CDR, complementarity determining region; ELISA, enzyme-linked immunosorbent assay; Fv, fragment of antibody variable region; IPTG, isopropyl β-D-thiogalactopyranoside; PCR, Polymerase Chain Reaction; scFv, single-chain Fv fragment; SOE-PCR, splicing by overlap extension PCR; TES, 0.2 M Tris-HCl, pH 8.0/0.5 M EDTA/0.5 M sucrose; VH, heavy chain variable domain; VL, light chain variable domain.

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