Short communication

A scFv phage display mini library generated from the immunoglobulin repertoire of breast medullary carcinoma infiltrating B lymphocytes

B. Kotlan[a,b,*], P. Simsa[c], N. Gruel[d], J. Foldi[a], W.H. Fridman[b], G. Petranyi[a] and J.L. Teillaud[d]
[a]*National Institute of Haematology and Immunology, Budapest, Hungary*
[b]*INSERM U255, Paris, France*
[c]*Human Co. Ltd., Godollo, Hungary*
[d]*Laboratoire de Biotechnologie des Anticorps, Paris, France*

1. Introduction

The detection and characterization of antigens expressed during the progression of carcinogenesis is still a major goal for tumour immunology. We describe a possible new way for defining human tumour specific antigens with the use of scFv phage display technology [1]. An attractive hypothesis is that tumour infiltrating lymphocytes (TIL) are accumulated in the tumour tissue because of their unique capacity for recognizing tumour cells [2]. This has been proved in the case of tumour infiltrating T lymphocytes (TIL-T) [3, 4]. In contrast, knowledge about tumour infiltrating B lymphocytes (TIL-B) is very limited [5–7].

In this work we show data on the immunoglobulin repertoire expressed by TIL-B cells from medullary breast carcinoma (MBC). A detailed DNA sequence analysis of immunoglobulin heavy (VH) and light chain (VL) variable region genes was performed. A single chain Fv phage display mini-library was generated from the selected VH and VL genes.

2. Materials and methods

The immunoglobulin repertoire analysis from a human medullary breast carcinoma was described in a previous paper using DNA sequence data analysis software (BLAST, BIOEDT) and databases accessible through the Internet (Kabat NIH, IMGT, Genbank) [8]. 26 VH, 32 Vκ and 21 Vλ clones were then selected for random VH/VL combination with a linker peptide [(Gly4Ser)3] for the construction of a scFv library. A ScFv phage display mini library was then generated and screened according to the method described by Gruel et al. [9].

3. Results

3.1. TIL-B immunoglobulin repertoire analysis

Subgroup analysis using the Kabat database showed that several subgroups of heavy and light chain variable regions were expressed by TIL-B cells of MBC. 26 VH-JH, 32 Vκ-Jκ and 21 Vλ-Jλ clones were randomly selected from 200 clones, sequenced, and compared (Table 1). Most of the VH sequences belong to subgroup III, while the most frequent Vκ subgroups are subgroup I and IV, and subgroup III for the Vλ sequences. Based on multiple alignments and search for homology among the sequences belonging to a defined subgroup, we defined "clusters" of variable regions. Sequences that exhibited more than 95% homol-

*Correspondence to: Beatrix Kotlan, National Institute of Haematology and Immunology, Budapest 1113, Daroczi Street 24, P.O.B. H-1519, Hungary. Fax: +36 1 4667 020; E-mail: kotlan@enzim.hu.

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Table 1
Subgroup analysis and homology levels of cloned VH and VL sequences

Ig variable region	Subgroup[a] / Sequences	Cluster[b] / Sequences	Homology
VH	I / 5	Ia / 3	95%–100%
		Ib / 2	95%–100%
	II / 5	IIa / 2	98%–100%
		IIb / 3	95%–100%
	III / 16	IIIa / 5	97%–100%
		IIIb / 5	97%–100%
		IIIc / 3	99%–100%
		3[c]	85%–94%
Vκ	I / 11	Ib / 6	95%–100%
		Ib / 2	95%–100%
		3[c]	85%–94%
	II / 7	IIa / 7	95%–100%
	III / 5	IIIa / 3	95%–100%
		2[c]	85%–94%
	IV / 9	IVa / 4	97%–100%
		IVb / 5	96%–100%
Vλ	I / 6	Ia / 3	95%–100%
		3[c]	85%–94%
	II / 5	IIa / 5	95%–100%
	III / 10	IIIa / 7	97%–100%
		3[c]	85%–94%

[a] Subgroups according to the Kabat/NIH database.
[b] Clusters defined as variable regions with 95–100% homology.
[c] Sequences that could not be clustered (because $<$ 95% homology).

ogy were grouped together. The five VH I and VH II sequences were divided in two clusters each and the 16 VH III sequences into three clusters (Table 1). In the VκI and VκIV subgroups, two clusters could be defined. All seven sequences belonging to VκII, and three out of five VκIII sequences showed homology greater than 95%. A similar high level of homology was observed among Vλ sequences, particularly in the VλII and VλIII subgroups.

3.2. Generation of scFv-phage display

Assembly of the selected rearranged immunoglobulin variable region heavy and light chain genes (26 VH, 32 Vκ and 21 Vλ was carried out by a three step PCR amplification, using a linker peptide (Gly4Ser) 3 coding sequence. The generated ScFv VK, and ScFv VL libraries, expressed in pHEN1, are currently being used to identify scFv directed to tumour-associated molecules.

4. Discussion and conclusion

Our current knowledge of V region gene expression in the human is derived from three main sources: the fetal B lymphocyte repertoire, B cell malignancies and autoantibodies stemming from a variety of autoimmune diseases, while the "normal adult" repertoire is relatively unexplored [10,11]. There are no data available about the immunoglobulin repertoire and clonality of B lymphocytes infiltrating solid tumours. Analysis showed that in this tumour, B cells express a polyclonal repertoire with some over-represented Ig VH and VL regions that may reflect an ongoing immune response against tumour cells. Further studies of the sequenced clones and comparison with germline sequences, to reveal the frequency of mutations, that should demonstrate whether antigen driven selection does exist.

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