Quantitative and qualitative assessment of anti-tumour specific T cells in patients with lung cancer and cancer free individuals

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We measured, in patients with lung carcinomas and healthy individuals, the frequency and qualitative characteristics of circulating precursor cytotoxic T lymphocytes (pCTLs) specific for naturally processed and presenting peptides of human telomerase reverse transcriptase (hTERT), MAGE-A1 and MAGE-A3. Their magnitude as well as the functional properties of isolated T cell clones have not been thoroughly evaluated in lung cancer, despite these peptides being used for the design of immunotherapeutic protocols.

Materials

Patients and normal donors (HLA-A1, A2, A24 and/ or B35)

20 patients with primary lung cancer
14 SCLC
5 ADC
1 NSCLC

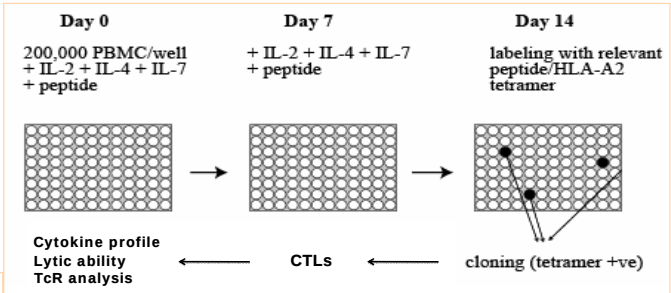
5 aged matched healthy male individuals

Antigen	Peptide	HLA-multimer
MAGE-A1	EADPTGHSY	A1-PE
MAGE-A1	EADPTGHSY	B35-APC
MAGE-A3	EVDPIGHLY	A1-PE
MAGE-A3	FLWGPRALV	A2-PE
MAGE-A3	AYACNTSTL	A24-PE
MAGE-A3	EVDPIGHLY	B35-PE

Antigen	Peptide	HLA-multimer
hTERT	ILAKFLHWL	A2-PE
hTERT	RLFFYRKSV	A2-PE
hTERT	VYAETKHFL	A24-PE
PB1	VSDGGPNLY	A1-APC
BMLF1	GLCTLVAML	A2-APC
EBNA3C	RYSIFFDYM	A24-APC

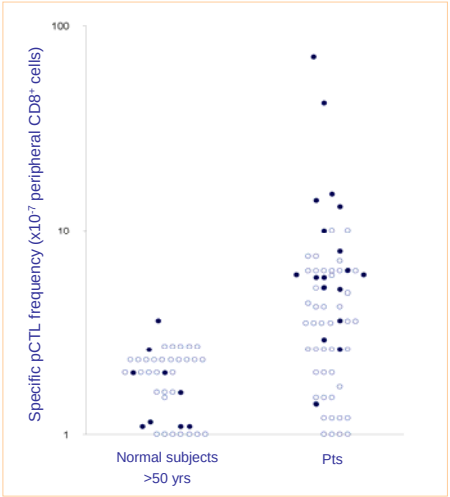
Methods

The frequency of the peripheral blood peptide-specific pCTLs was estimated using the most sensitive amongst the available methods that combines HLA-multimer flow cytometric technology with a previous step of *in vitro* amplification under limiting conditions [1, 2]. Peptides of viral origin were used as controls. Tetramer positive populations were detected, sorted and clones generated. The latter were studied with respect to their TcR, cytokine profile, phenotype and anti-tumour specific lytic ability (figure on right).

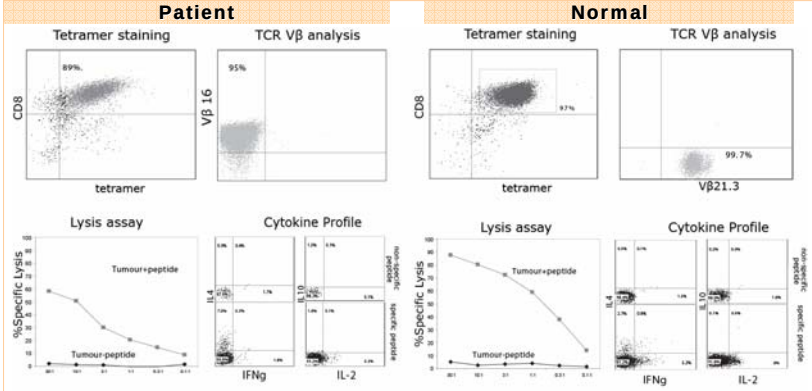


Schematic representation of MLPC

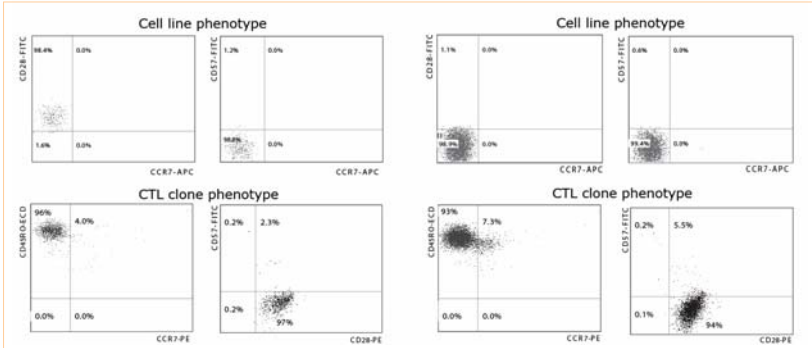
Results



Frequency of circulating pCTLs for the different peptides per 10^7 peripheral blood $CD8^+$ T cells. The estimated frequency of pCTL against all tumour peptides studied, was higher in patients than cancer-free individuals. Blue dots represent the detected pCTL frequency. White dots represent the minimum pCTL frequency determined on the basis of the maximum number of $CD8^+$ cells obtained from the patients, and amongst which, one specific pCTL could not be found. This designation was used to declare that the presence of specific pCTLs at lower frequencies cannot be excluded.



Characterization of isolated anti-tumour specific T cells in a lung cancer patient and a normal individual. T cell clones generated were analysed with respect to their Vb TcR and clonality. Both types of clones, did not display any different characteristics with respect to their lytic capacity against tumour cells and their ability to specifically secrete cytokines.

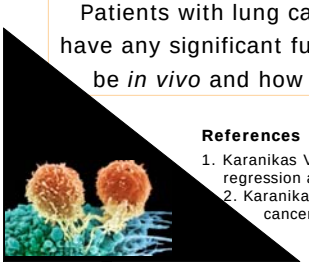


Phenotypic characterization of peptide specific tetramer positive T cell populations revealed that lines obtained from cancer patients expressed CD28 on their surface.

CD8 T cell clones obtained from both patients and normal individuals were $CD8^+$ $CD45RO^+$ $CCR7^-$ $CD28^+$ $CD57^-$.

Conclusion

Patients with lung cancer and healthy individuals contain in their blood anti-tumour specific CTL clones. These do not appear to have any significant functional difference, apart from a possible alteration in their phenotype, questioning what their function might be *in vivo* and how amplifying them by the application of various immunotherapeutic protocols could benefit patient outcome.



References

1. Karanikas V et al. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant canarypox virus. *J Immunol* 2003, 171:4898-904
2. Karanikas V et al. Low-frequency CD8+ T cell precursors specific for survivin and survivin-2B in cancer patients: A caveat for immunotherapy? *Cancer Immunology and Immunotherapy* 2007 (submitted)

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