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Human tumor-specific T lymphocytes: does function matter more than number?

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In recent years, several clinical trials have involved the vaccination of cancer patients with tumor-specific antigens that are recognized by T lymphocytes. Anti-vaccine T-cell responses in these patients have been monitored on the assumption that their magnitude would correlate with clinical efficacy. Although analysis of these data show that such a correlation is emerging, detailed analyses of the few patients who benefit clinically from the vaccinations suggest that the function of the anti-vaccine T cells might be more important than their number. Recent studies show that in cancer patients numerous tumor-specific T cells appear to be quiescent in the presence of the tumor. Understanding how an efficient vaccine interferes with this coexistence is one of the current challenges of cancer immunotherapy.

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Current Opinion in Immunology 2005, **17**:320–325

This review comes from a themed issue on
Lymphocyte effector functions
Edited by Lorenzo Moretta

Available online 11th April 2005

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2005.03.002

Introduction

Human T lymphocytes that recognize tumor-specific antigens have become amenable to precise immunological analysis as a result of the identification of a wide variety of tumor-specific antigens and the development of tetramer technology. Clinical trials using vaccines comprising well-defined tumor antigens are usually followed by an analysis of the anti-vaccine T-cell response in search of a meaningful surrogate marker of clinical efficacy. But even though several methods can be used to estimate the frequencies of human anti-vaccine T cells, only few studies have tried to establish a correlation between the detection of T-cell responses and clinical outcomes in vaccinated patients. The first part of this review summarizes these studies; the second part of this review focuses on the notion that high frequencies of tumor-specific T cells do not guarantee therapeutic efficacy, and that the function of these lymphocytes could matter more than their number.

Before discussing the numbers and functions of tumor-specific T cells in the context of therapeutic vaccination, it is important to remember that an optimal T-cell response will not lead to rejection of a tumor that has become resistant to immune attack because of loss of antigen expression or other mechanisms. In a recent clinical trial combining conditioning chemotherapy, transfer of anti-tumor T cells and IL-2 therapy, 18 out of 35 melanoma patients experienced a clinical response [1•]. This observation indicates that at least 50% of melanoma tumors cannot completely resist immune attack.

Frequency of tumor-specific anti-vaccine T cells: does number count?

Clinical studies of anti-tumoral vaccination have been monitored on the premise that massive anti-vaccine T-cell responses are required for tumor rejection. In mice vaccinated with tumor antigens, the intensity of anti-vaccine T-cell responses appears to correlate with clinical efficacy [2,3]. Is this also observed in patients?

Estimating the frequency of human tumor-specific T cells

Only a few methods are available to estimate the frequencies of T cells that recognize particular defined tumor antigens. A direct estimation of this frequency can be obtained using *ex vivo* assays such as tetramer labeling or cytokine secretion measured by Elispot, provided that the frequency exceeds a threshold of about 5×10^{-4} of the $CD4^+$ or $CD8^+$ T cells present. To detect T cells at lower frequencies the lymphocytes have to be amplified first by re-stimulation *in vitro* with the antigen. This results in two difficulties. Firstly, a frequency can only be estimated if re-stimulation is carried out in limiting dilution conditions, which involves a heavy workload. Secondly, you can only measure the frequency of those precursors that proliferated enough to generate a detectable clonal progeny. This leads to underestimated frequencies.

It is noteworthy that the absence of detectable T cells in the *ex vivo* assays does not exclude the possibility that a response occurred *in vivo*. The reason for this is that the detection threshold of 5×10^{-4} necessary for the *ex vivo* assays is 1 000-fold higher than the frequency of naïve T cells. For T cells recognizing peptide MAGE-$A3_{168–176}$ on HLA-A1 this frequency is 4×10^{-7} of $CD8^+$ cells [4], and

we observed similar frequencies for T cells that recognize gp100, NA17, LAGE-1, or MAGE-A10 antigens. The Melan-$A^{MART\text{-}1}{}_{28–36}$ peptide is a remarkable exception, with a very high naïve T cell frequency of about 5×10^{-4} of $CD8^+$ cells [5].

Frequencies of anti-vaccine T cells

Reported frequencies of anti-vaccine T cells following immunization with tumor antigens vary from $>10^{-2}$ to 10^{-6} of the total T-cell population. Anti-Melan-$A^{MART\text{-}1}$ T cells were found at frequencies of up to 2×10^{-2} of $CD8^+$ cells in melanoma patients vaccinated with peptide either in incomplete Freund's adjuvant (IFA) [6], or in IFA with CpG [7]. Anti-$gp100_{209–217}$ cytotoxic T lymphocytes (CTLs) were found at $>10^{-2}$ of $CD8^+$ cells in tumor-free melanoma patients vaccinated with peptide in IFA [8,9]. Anti-MAGE-$A3_{168–176}$ CTLs were present at 3×10^{-3} and 10^{-3} in patients vaccinated with ALVAC-MAGE or peptide-pulsed dendritic cells (DCs), respectively [10,11]. HLA-DP4-restricted $CD4^+$ cells recognizing peptide MAGE-$A3_{243–258}$ were found at 7×10^{-4} of $CD4^+$ cells after vaccination with peptide-pulsed DCs [12]. Finally, several patients vaccinated with peptide or ALVAC-MAGE had monoclonal anti-MAGE-$A3_{168–176}$ CTL responses at low frequencies of about 10^{-6} of $CD8^+$ cells [4,10].

T-cell responses and clinical outcomes

Very few studies have analyzed whether these anti-vaccine T-cell responses correlate with the observed tumor regressions [4,6,13,14] (Table 1). A correlation seems to emerge from two studies [4,6], compatible with the hypothesis that the anti-vaccine T-cell response is necessary, but not in itself sufficient, to initiate tumor rejection. A tight correlation is unlikely to be found for two reasons. First, some patients have strong anti-vaccine T-cell responses without detectable clinical benefit. It is certain that a limiting factor for clinical efficacy, in addition to the frequency of anti-vaccine T cells, is tumor resistance to immune attack. Second, and perhaps more surprisingly, some patients display tumor regression with no or very few detectable anti-vaccine T cells [4]. In such patients, tumor-specific CTLs that recognized antigens absent from the vaccine were primed or amplified after vaccination [15•]. In regressing metastases, these anti-tumor CTLs were 10 000 times more frequent than the anti-vaccine T cells and, therefore, probably effected tumor rejection [16•]. These results are in line with those of other groups that described post-vaccination T cells which recognized tumor antigens that were absent from the vaccine [17–19]. A plausible model is that anti-vaccine T cells, even at very low frequencies, modify an immunosuppressive environment within the tumor, opening a permissive window for the priming or re-stimulation of other anti-tumor T cells.

Functions of anti-tumor T cells: what is involved?

High frequencies of anti-tumor T cells, present either after vaccination [6] or after spontaneous anti-tumor responses [15•], do not secure tumor regression. The coexistence of tumor cells and primed tumor-specific T

Table 1

Studies addressing the correlation between immunological and clinical responses in metastatic melanoma patients with detectable disease and vaccinated with defined tumor antigens.

Vaccines	Antigenic peptides	Patients displaying regression of ≥1 metastasis	Method of monitoring anti-vaccine T cells	Reported T cell responses in patients with:		Ref.
				Evidence of tumor regression	No evidence of tumor regression	
ALVAC-MAGE[a]	MAGE-$A3_{168–176}$ (HLA-A*0101)	4/15	MLPC-tetramer cloning[d]	3/4	1/11	[4]
Peptide + IFA	Melan-$A_{28–36}$ (HLA-A*0201)	2/21	*ex vivo* tetramer/ *ex vivo* elispot IFNγ	2/2	4/19	[6]
Mono-DC[b] + peptide	MAGE-$A3_{168–176}$ (HLA-A*0101)	6/11	*ex vivo* elispot IFNγ	5/6	4/5	[13]
CD34-DC[c] + peptide	Melan-$A_{28–36}$, $tyrosinase_{368–376}$, $gp100_{g209–2\ M}$, MAGE-$A3_{271–279}$ (HLA-A*0201)	7/18	*ex vivo* elispot IFNγ	7/7[e]	9/11[5]	[14]

[a]ALVAC-MAGE is a recombinant canarypox virus of the ALVAC type carrying a minigene coding for two antigenic peptides: MAGE-A3168–176 and MAGE-A1161–169. [b]Dendritic cells derived from adherent blood mononuclear cells cultured with GM-CSF and IL-4 and matured by monocyte-conditioned medium. [c]Dendritic cells derived from circulating CD34+ precursor cells mobilized by G-CSF, cultured with GM-CSF, FLT3-L and TNF. [d]Mixed lymphocyte–peptide cultures in which blood mononuclear cells are stimulated with peptide over two weeks, followed by labeling with tetramer. Anti-vaccine CTL clones are cloned from the tetramer-positive cells. [e]In this study, 6/7 clinical regressors and 3/11 clinical progressors responded to at least three of the four antigens. Abbreviations: FLT3-L, fms-related tyrosine kinase 3 ligand; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor.