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Relapse or Eradication of Cancer Is Predicted by Peptide-Major Histocompatibility Complex Affinity

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SUMMARY

Cancers often relapse after adoptive therapy, even though specific T cells kill cells from the same cancer efficiently in vitro. We found that tumor eradication by T cells required high affinities of the targeted peptides for major histocompatibility complex (MHC) class I. Affinities of at least 10 nM were required for relapse-free regression. Only high-affinity peptide-MHC interactions led to efficient cross-presentation of antigen, thereby stimulating cognate T cells to secrete cytokines. These findings highlight the importance of targeting peptides with high affinity for MHC class I when designing T cell-based immunotherapy.

INTRODUCTION

Relapse of cancers is very common, even following combinatorial therapy of surgery, chemotherapy, radiation, and/or immunotherapy. For maximal efficacy, drugs depend on reaching the necessary concentration in the tumor microenvironment (Skipper, 1986). This critical concentration concept also applies to cellular effectors, such as neutrophils and T cells (Budhu et al., 2010; Li et al., 2002, 2004). While cellular effectors or drugs at optimal concentrations can eradicate all sensitive cancer cells, relapse may still occur because of the outgrowth of variants. Cancer cells show extremely high genetic instability, and cancers always contain variants that are resistant to destruction by a particular drug or T cell (Anders et al., 2011; Hanson et al., 2000), very similar to what is found for viruses (Hensley et al., 2009).

For complete eradication, it is important to eliminate every residual cancer cell, including heritable variants (Singh et al., 1992; Spiotto et al., 2004; Zhang et al., 2007). However, factors responsible for T cell elimination of variants have not been determined. In experiments designed to explore the reason for failed T cell treatment, we took a reductionist approach, ultimately directing our focus to the target peptides and, in particular, to their affinities for major histocompatibility complex (MHC) class I. We selected several peptides that, when targeted, caused tumor eradication and others that caused relapse. To reduce the influence of differences between cancers, we used two cancer cell lines that were both transduced to express the different peptides. To reduce differences due to expression levels, we used the same design of triple peptides fused to fluorescent proteins. Proteasomal cleavage of proteins may not generate (Chapiro et al., 2006; Popovic et al., 2011) or destroy immunogenic peptides (Schultz et al., 2002). To minimize differences in proteasomal cleavage of the fusion proteins, we designed peptide triplets separated by "Ala-Ala-Tyr" cleavage sites. We targeted antigens with no known oncogenic activity to reduce the possibility that the nature of a particular targeted antigen prevented the cancer from escaping. To exclude the influence of other T cells helping or regulating the relevant $CD8^+$ T cells, T cell receptor (TCR)-transgenic T cells with a single specificity were adoptively transferred into hosts, which were TCR-transgenic for an irrelevant target. Finally, a single adoptive T cell transfer regimen was used without providing any additional stimulation, such as vaccination or administration of cytokine.

RESULTS

Cancer Cells Expressing Different Peptides Are Killed by T Cells with Similar Efficacy In Vitro

EGFP was fused to minigenes encoding the peptides hen egg ovalbumin$_{257–264}$ (OVA$_{257}$), model peptide SIYRYYGL

Significance

Cancer relapse remains the greatest obstacle to virtually any cancer therapy. Our data show that high affinity of the targeted peptides for MHC is required for strong stimulation of T cells to secrete cytokines and cause relapse-free tumor eradication. Adoptive T cell transfer therapies should, therefore, target peptides that have high affinities for the presenting MHC class I.

Table 1. Abbreviations, Conditions, and Summary of Results for Key Experiments

Target Peptide on Cancer Cells				Hosts		T Cells		
Designation	Sequence	MHC	Affinity of Peptide for MHC (IC_{50} [nM])[a]	Designation	Relationship of Antigen to Recipient	Designation	Relationship of Antigen to Donor	Tumor Rejection
SIY	SIYRYYGL	K^b	1.1	OT-I	nonself	2C	nonself	5/5[b,c,d,e]
						none		0/6[b]
$OVA_{257–264}$	SIINFEKL		0.9	2C	nonself	OT-I	nonself	4/4[f]
						none		0/4[f]
$Tyr_{369–377}$	FMDGTMSQV	A2	4.2[g]	OTA	self	FH	self	6/7[h]
						none		0/5[h]
$hgp100_{25–33}$	KVPRNQDWL[i]	D^b	186	OT-I	nonself	pmel	nonself	1/8[c]
						none		0/2
EGP	EGPRNQDWL		454	OT-I	nonself	pmel	nonself	1/6[d]
						none		0/5
$mgp100_{25–33}$	EGSRNQDWL		22,975	OT-I	self	pmel	self	1/12[e]
						none		0/6

See Table S1 for details.
[a] IC_{50} values represent the geometric mean of five or more experiments.
[b] $p = 0.002$.
[c] $p < 0.005$.
[d] $p = 0.015$.
[e] $p < 0.001$.
[f] $p < 0.029$.
[g] A higher IC_{50} value of 65 nM was published for this peptide earlier (Colella et al., 2000). The differences in affinity measurements likely arose as a result of small differences in reagents, methodology, and procedures.
[h] $p = 0.015$.
[i] Only the underlined amino acids differ between the three gp100 peptide variants.

(SIY), mouse $Tyrosinase_{369–377}$ (Tyr_{369}), mouse or human $gp100_{25–33}$ ($mgp100_{25}$ and $hgp100_{25}$, respectively), and a heteroclitic $gp100_{25–33}$ (EGP); EGP differs from $mgp100_{25}$ only in the third amino acid (EGPRNQDWL versus EGSRNQDWL), while it shares the proline at position 3 with $hgp100_{25}$ (KVPRNQDWL) (Table 1). A Cerulean fusion gene was generated only for SIY (Figures 1A and 1C, top). The fibrosarcoma line MC57 of C57BL/6 origin was used to generate lines that expressed the fusion genes at high levels (Figure 1B). Furthermore, the chimeric human leukocyte antigen (HLA)-A2.1/H-2D^b molecule HHD was cotransduced with the Tyr_{369}-EGFP fusion protein to generate MC57-TyrHHD (Figure 1C, bottom).

Assays in vitro demonstrated similar killing of the cancer lines by cognate peptide-activated T cells (Figure 1D). 2C T cells, whose TCR binds SIY, killed the MC57-SIY line, and pmel T cells killed MC57 cells expressing mouse $gp100_{25}$, human $gp100_{25}$ or EGP. Interestingly, Tyr_{369}-specific T cells derived from the *FH* TCR-transgenic, tyrosinase (*Tyr*)-deficient albino mouse (AFH) or *Tyr*-positive black mouse (FH) killed MC57-TyrHHD target cells similarly well. Together, the results imply there is sufficient direct presentation of all processed peptides and sufficient avidity of the T cells for efficient killing in vitro.

T Cells Targeting SIY, OVA_{257}, or Tyr_{369} Eradicate Large Tumors

SIY-expressing MC57-SIY cells were injected in TCR-transgenic mice of irrelevant specificity (OT-I). OVA_{257}-transfected cancer cells were injected in *2C* TCR-transgenic mice; MC57-TyrHHD cancer cells were grown in *OT-I* TCR- and *AAD*-transgenic mice, which did (OTA) or did not express tyrosinase (albino; AOTA). In all cases, cancer cells produced progressively growing tumors within 1 week (Figure 2A). At least 2 weeks after cancer cell injection, when tumors reached about 500 mm^3, mice were treated with T cells. As published by our laboratory, tumors expressing SIY and treated with 2C T cells were eradicated (Figure 2B, upper left; Table 1; Table S1 available online; Spiotto et al., 2004). Here, we also show that OVA_{257}-expressing tumors treated with OT-I T cells were rejected (Figure 2B, middle left) and FH T cells eradicated Tyr-positive tumors (Figure 2B, lower left). In this last experiment, FH T cells derived from a *Tyr*-positive donor were transferred into a *Tyr*-positive host and eradicated a Tyr-expressing tumor. Together, this and other experiments using *FH* TCR-transgenic T cells from *Tyr*-negative donors (AFH) and *Tyr*-negative (AOTA) or *Tyr*-positive (OTA) hosts showed that tumors could be rejected (1) whether the targeted peptide was self or nonself for the tumor-bearing host and (2) whether the targeted peptide was self or nonself for the donor T cells (Figure S2A). This may be unique to our model, since a different model showed that low ubiquitous expression of a transgene prevented the rejection of antigen-expressing tumors through the induction of tolerance (Buschow et al., 2010). Levels of antigen expression in the host and/or tumor, type of cells that express the self-antigen, and the source of T cells may likely influence the outcome. Taken together, targeting any of the three peptides, SIY, OVA_{257}, or Tyr_{369}, caused eradication of established large and solid tumors.