Ex vivo induction and expansion of antigen-specific cytotoxic T cells by HLA-Ig–coated artificial antigen-presenting cells

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Adoptive immunotherapy holds promise as a treatment for cancer and infectious diseases, but its development has been impeded by the lack of reproducible methods for generating therapeutic numbers of antigen-specific CD8+ cytotoxic T lymphocytes (CTLs). As a result, there are only limited reports of expansion of antigen-specific CTLs to the levels required for clinical therapy. To address this issue, artificial antigen-presenting cells (aAPCs) were made by coupling a soluble human leukocyte antigen–immunoglobulin fusion protein (HLA-Ig) and CD28-specific antibody to beads. HLA-Ig–based aAPCs were used to induce and expand CTLs specific for cytomegalovirus (CMV) or melanoma. aAPC-induced cultures showed robust antigen-specific CTL expansion over successive rounds of stimulation, resulting in the generation of clinically relevant antigen-specific CTLs that recognized endogenous antigen–major histocompatibility complex complexes presented on melanoma cells. These studies show the value of HLA-Ig–based aAPCs for reproducible expansion of disease-specific CTLs for clinical approaches to adoptive immunotherapy.

Adoptive immunotherapy with antigen-specific, antiviral CTLs was first successfully performed in patients using *ex vivo* expanded CMV-specific CTL clones as prophylaxis for CMV disease in immunocompromised allogeneic bone marrow transplant recipients[1]. Similarly, adoptively transferred *ex vivo* expanded CTLs have had encouraging, albeit limited, success in the treatment of Epstein-Barr virus and melanoma[2,3]. Development of adoptive immunotherapy has been impeded by the lack of a reproducible, economically viable method to generate therapeutic numbers of antigen-specific CTLs. A current standard approach to generating antigen-specific CTLs entails generating monocyte-derived dendritic cells (DCs) for expansion of CTLs. This step is both time-consuming and expensive. Use of DCs for CTL expansion to clinically relevant amounts of CTLs requires multiple leukophoreses to obtain enough autologous DCs. Variability in both the quantity and quality of DCs obtained, which presumably relates to underlying disease and patient pre-treatment, also significantly impacts on the viability of DC-based *ex vivo* therapeutics. For these reasons, use of DCs has been a limiting step in *ex vivo* expansion of T cells.

Other approaches for expansion of antigen-specific CTLs from enriched populations have used nonspecific techniques based on CD3-specific antibodies[4]. There are two problems with these techniques, however. First, beads with antibodies against CD3 and CD28 support long-term growth of CD4+ T cells but do not sustain long-term growth of CD8+ T cells[5]. In addition, approaches using stimulation with CD3-specific antibodies are associated with a decrease in antigenic specificity, even when starting with highly enriched antigen-specific CTL populations[6].

Soluble forms of HLA molecules loaded with antigen-specific peptides are able to directly target cognate CD8+ T cells *in vitro*[7] and may be useful in generating aAPCs. aAPCs based on immobilized, soluble HLA complexes could potentially overcome the limitations associated with approaches based on both DCs and CD3-specific antibodies. Antigen-HLA complexes, along with the appropriate co-stimulatory molecules, may offer a viable method to expand antigen-specific T cells for therapeutic use. Here, we immobilized a dimeric form of HLA (HLA-Ig), together with CD28-specific antibody onto beads. HLA-Ig, in which HLA has been fused to an immunoglobulin molecular scaffold, takes advantage of the flexibility of the Ig portion to increase the apparent T-cell receptor binding affinity. Using this approach, we have developed an aAPC that reproducibly stimulates robust CTL expansion and can be easily manufactured. We show that HLA-Ig–based aAPCs induced and expanded antigen-specific CTLs *in vitro* to both immunodominant and subdominant epitopes from normal healthy donors, as well as from a patient with melanoma. aAPC-induced antigen-specific CTLs also recognized endogenously processed antigen presented on target cells. The *ex vivo* adoptive immunotherapy platform technology developed here may complement other active immunotherapy regimens.

Induction of Mart-1– and CMV-specific CTLs by aAPCs

Two clinically relevant targets, CMV and melanoma, were chosen to show proof of principle. Peptides derived from either CMV or the melanoma-associated antigen, Mart-1, when presented by HLA-A2, have widely varying affinities for their cognate T-cell receptor. The CMV peptide pp65 is known to be a high-affinity peptide, whereas the modified Mart-1 peptide[8], derived from a melanocyte self antigen, is a low-affinity peptide. Thus, studying induction and expansion in these two systems by HLA-Ig–based aAPCs provides insight into the potential therapeutic value of HLA-Ig–based aAPCs.

Current approaches use autologous peptide-pulsed DCs to induce antigen-specific CTLs from normal peripheral blood mononuclear cells (PBMCs; Fig. 1). These approaches often use DC- or CD40L-stimulated autologous B cells to induce antigen-specific CTLs[9,10] over two to four stimulation cycles (Fig. 1, step
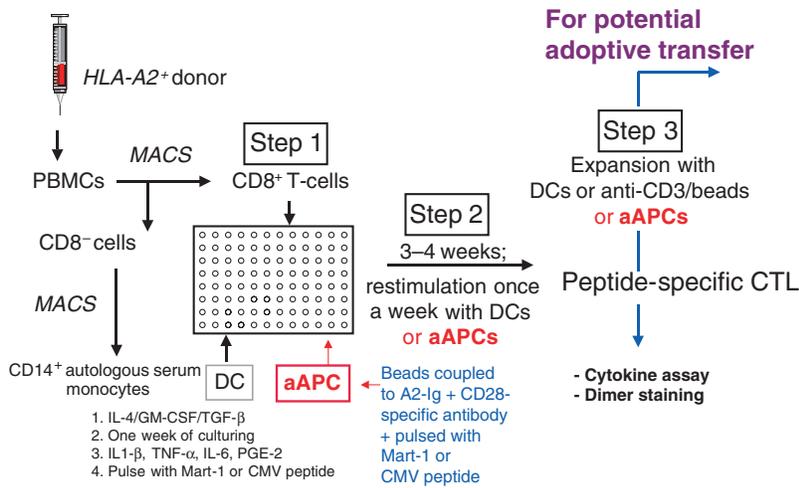
Fig. 1 Schematic of induction and expansion of peptide-specific CTLs by autologous DCs or aAPCs. Step 1 (induction phase): enriched CD8+ T cells are co-cultured with peptide-pulsed, autologous monocyte-derived DCs or with peptide-pulsed aAPCs in 96-well plates. Step 2 (enrichment and small-scale expansion phase): CD8+ T cells are collected, old aAPCs are removed and T cells are restimulated with freshly generated peptide-pulsed autologous DCs or with new peptide-pulsed aAPCs. Step 3 (large-scale expansion phase): antigen-induced CTLs are expanded either specifically with peptide-pulsed aAPCs or non-specifically with CD3-CD28 antibody beads.

single round of stimulation up to 90% of the CTL population was CMV-specific using aAPCs. Slightly fewer CMV-specific CTLs were seen using DCs. Thus, aAPCs were generally as effective as, if not better than, DCs at inducing antigen-specific CTLs in two different CTL systems from multiple healthy donors and from a patient with melanoma.

aAPCs also mediated substantial expansion of CTLs specific for the A2-restricted subdominant melanoma epitope NY-ESO-1 (ref. 11) and the subdominant Epstein-Barr virus epitope derived from LMP-2 (ref. 12; see Supplementary Table 1 online). Approximately 1.2% of all CD8+ cells were NY-ESO-1–specific after three rounds of aAPC stimulation. Although this is clearly lower than seen in expansion of CTLs specific for immunodominant epitopes, lower numbers of antigen-specific CTLs are expected when analyzing expansion of CTLs specific for subdominant epitopes. NY-ESO-1–specific CTLs mediated lysis of cognate-specific target cells but not irrelevant target cells (see Supplementary Table 1 online). In contrast, DC-based stimulation resulted in a substantially lower frequency of NY-ESO-1–specific CTLs without detectable cytotoxic activity in a standard ^{51}Cr-release assay.

**Recognition of endogenously processed antigen**
An important criterion in evaluating CTL function is recognition of targets expressing endogenous antigen-HLA complexes.

2) until the antigen-specific CTLs become a prominent part of the culture. We therefore compared aAPC induction to induction by DCs. After three rounds of stimulation with MART-1 peptide-loaded aAPCs, 62.3% of all CD8+ CTLs were Mart-1–specific, as determined by staining with HLA-A2–Ig dimer loaded with the Mart-1 peptide ($^{Mart-1}$A2-Ig, Fig. 2a, left) and 84.3% as determined by intracellular cytokine staining (Fig. 2a, right). In contrast, autologous DCs induced only 29.7% MART-1–specific cells by dimer staining and 61.1% by intracellular cytokine staining of Mart-1–specific CTLs (Fig. 2b).

To explore the growth potential of aAPC-stimulated PBMCs, T cells were stimulated with aAPCs for seven weeks. Starting from 1×10^6 total CD8+ T cells that were less than 0.05% Mart-1–specific, cells expanded to approximately 10^9 CTLs that were greater than 85% antigen-specific (Fig. 2c and d). This represents at least a 10^6-fold expansion of antigen-specific cells in less than two months.

aAPC-mediated stimulation was as effective as, if not better than, DC stimulation of both Mart-1– and CMV-induced CTLs (Table 1). This was seen in four of five experiments using three different healthy donors and a patient with metastatic melanoma for Mart-1–loaded aAPCs, and from three different donors for CMV-loaded aAPCs. For Mart-1 induction, aAPCs induced about two- to three-fold more antigen-specific cells than with DCs, as seen with both HLA-Ig dimer staining and intracellular cytokine staining. This was also seen in a patient with metastatic melanoma (donor no. 7; Table 1). Induction of CMV-specific CTLs was more robust than that of Mart-1–specific CTLs, as even after a

Table 1 Comparison of Mart-1– and CMV-specific CTLs induced by aAPCs or DCs

Donor	Stimulus	Cytokine assay (% positive)				Dimer staining (% positive)	
		T cells only	T2	CMV	MART-1	Unloaded A2-Ig	MART-1 –loaded A2-Ig
1A1	DC	ND	ND	ND	ND	0.1	13.5
1A1	aAPC	ND	ND	ND	ND	0.7	33.4
1A4	DC	0.2	0.6	0.4	13.2	1.5	14.4
1A4	aAPC	0.3	3.2	2.6	32.6	2	54
5A	DC	0.2	0.2	0.2	49.1	0.8	19.2
5A	aAPC	ND	ND	ND	ND	0.1	4
6A	DC	0	0.1	0.1	68.7	0.1	20.8
6A	aAPC	0	0	0	84.6	0.3	65.9
7A	DC	ND	ND	ND	ND	2.9	28.0
7A	aAPC	ND	ND	ND	ND	0.2	79.5
						Unloaded A2-Ig	CMV -loaded A2-Ig
2B1	DC	ND	ND	ND	ND	1.7	58
2B1	aAPC	ND	ND	ND	ND	2.6	69
8B	DC	ND	ND	ND	ND	1.2	83.8
8B	aAPC	ND	ND	ND	ND	4.7	88.1
9B	DC	0.2	ND	93	0.2	2.3	98.5
9B	aAPC	0.3	0.3	82	0.2	0.6	92.1

Mart-1 specific CTLs were from donors 1, 5, 6 and 7; CMV-specific CTLs were from donors 2, 8 and 9. The frequency of antigen-specific CTLs was analyzed by dimer staining and intracellular cytokine staining. The percentage of peptide-specific CD8+ T cells is shown. ND, no data.
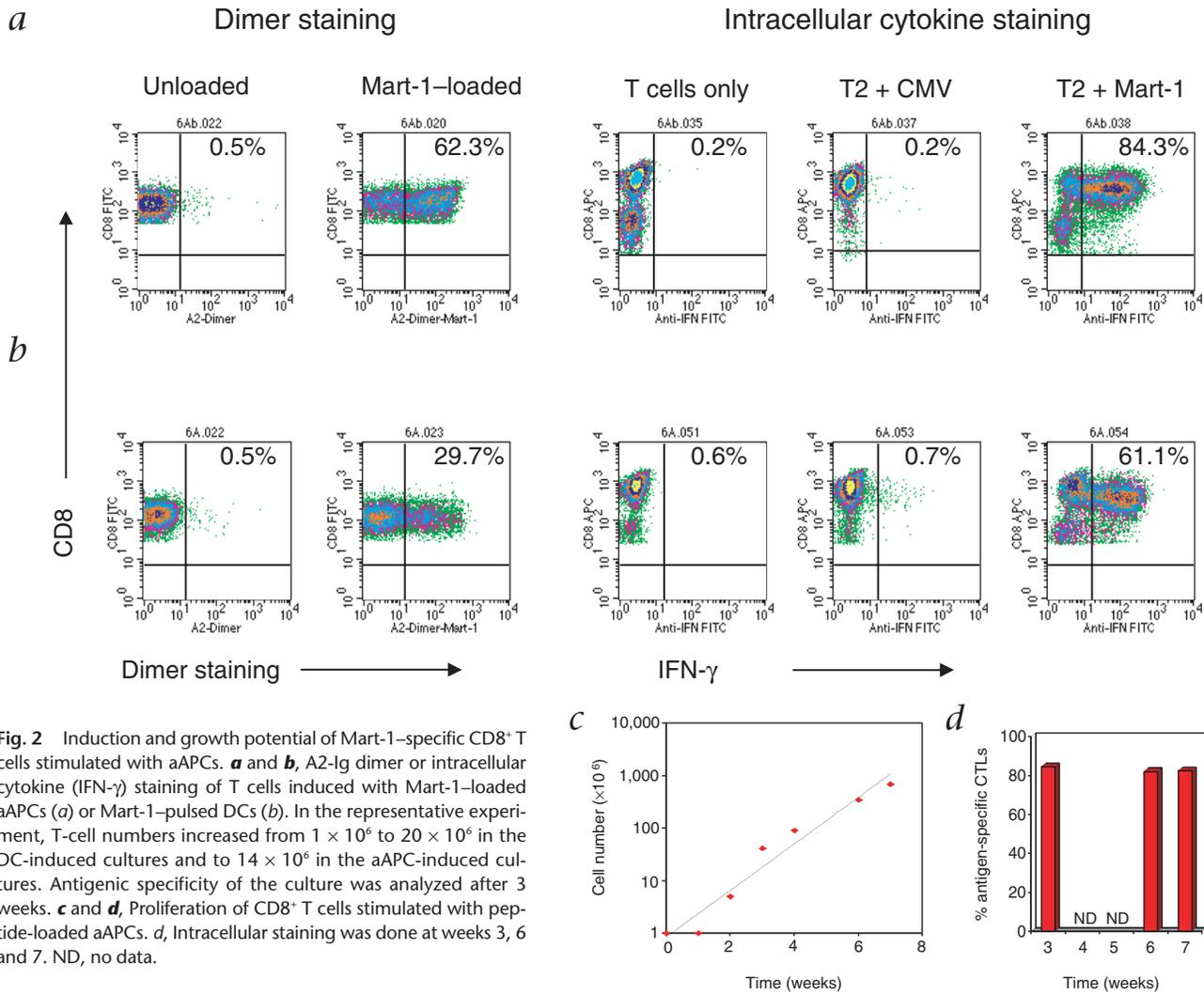
Fig. 2 Induction and growth potential of Mart-1–specific CD8^{+} T cells stimulated with aAPCs. **a** and **b**, A2-Ig dimer or intracellular cytokine (IFN-γ) staining of T cells induced with Mart-1–loaded aAPCs (*a*) or Mart-1–pulsed DCs (*b*). In the representative experiment, T-cell numbers increased from 1×10^6 to 20×10^6 in the DC-induced cultures and to 14×10^6 in the aAPC-induced cultures. Antigenic specificity of the culture was analyzed after 3 weeks. **c** and **d**, Proliferation of CD8^{+} T cells stimulated with peptide-loaded aAPCs. *d*, Intracellular staining was done at weeks 3, 6 and 7. ND, no data.

Initial work using peptide-pulsed DCs for expansion of melanoma-specific CTLs resulted in low-affinity CTLs that mediated lysis of targets pulsed by the cognate antigen, but often did not recognize melanoma targets that endogenously expressed antigen-HLA complexes[13]. We therefore studied the ability of aAPC-induced CTLs to recognize endogenous Mart-1 or CMV pp65 antigen (Fig. 3). When Mart-1–specific aAPC-induced cells were stimulated with melanoma target cells, ~37% produced IFN-γ (Fig. 3*a*). A comparable number made interleukin (IL)-4 (see Supplementary Fig. 1 online). A control Mart-1^{+}HLA-A2[−] melanoma target did not stimulate significant effector cytokine production. aAPC-induced effector CTL populations mediated dose-dependent lysis of target Mart-1^{+}HLA-A2[+] melanoma target cells but not control Mart-1^{+}HLA-A2[−] targets (Fig. 3*b*). aAPC-induced Mart-1–specific CTLs derived from PBMCs obtained from a patient with advanced melanoma were also able to mediate lysis of HLA-A2[+] Mart-1–expressing melanoma cells, with 14.7% lysis seen at an effector-to-target ratio of 25:1.

aAPCs were also able to induce CMV-specific CTLs that recognized endogenously processed and presented pp65 antigen (Fig. 3*c* and *d*). When stimulated with A293 cells transfected with pp65 (A293-N pp65[+] targets), ~45% of the cells produced IFN-γ. aAPC-induced effector CTL populations also mediated dose-dependent lysis of transfected target cells but not of control targets

(Fig. 3*d*). Thus, aAPC-induced CTL cultures from normal healthy donors, as well as from patients with melanoma, recognized endogenously processed antigen-HLA complexes.

Expansion of CMV-specific CTLs by aAPCs
One limitation associated with use of DCs is that expansion of CTLs to clinically relevant numbers requires either leukophoresis to obtain enough DCs or a non-specific expansion technique such as CD3-specific antibody beads (see Fig. 1, step 3). We therefore compared the 'expansion' phase using aAPCs or beads with antibodies against CD3 and CD28. During the expansion of CMV-specific CTLs, there was a seven-fold increase in the total number of CTLs using CD3-CD28 antibody beads. The percentage of antigen-specific cells, however, declined from 87.9% to 7.3% (compare Fig. 4*a* and *b*). This issue has limited the usefulness of using CD3-specific antibody–based expansion of diverse CTL populations[6]. In contrast, when CMV-specific aAPCs were used to expand antigen-specific CTLs, there was no concomitant loss of antigenic specificity. The percentage of CMV-specific CTLs remained over 86% (compare Fig. 4*a* and *c*) in those cultures and there was still a seven-fold increase in the number of T cells. Thus, HLA-Ig-based aAPCs support continued expansion of CTLs in an antigen-specific fashion and represent a substantial advance over CD3-specific antibody–based expansion.
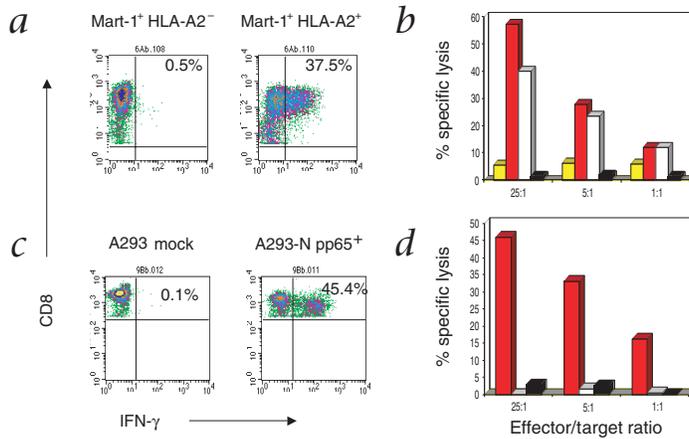
Fig. 3 aAPC-induced antigen-specific CTLs recognize endogenous melanoma or CMV pp65 antigen on target cells. ***a***, Intracellular cytokine staining of Mart-1–specific CD8+ T cells stimulated with a Mart-1+HLA-A2− (left) or Mart-1+HLA-A2+ (right) melanoma cell line. ***b***, Percent specific lysis by a Mart-1–specific CTL line for the following targets: T2 cells pulsed with non-cognate CMV peptide (▨), cognate Mart-1 peptide (▥), allogeneic HLA-A2+ Mart-1 melanoma cell line (□) or allogeneic HLA-A2− Mart-1 melanoma cell line (■). ***c***, Intracellular cytokine staining of CMV-specific CD8+ T cells stimulated with either pp65-negative control (left) or pp65-transfected (right) HLA-A2+ A293 cells. ***d***, Percent specific lysis by a CMV-specific CTL line is shown for the following targets: pp65-transfected A293 cells (▥), non-transfected HLA-A2+ A293 cells (□) or control CMV intermediate-early protein–transfected A293 cells (■). Values represent triplicate experiments at effector/target ratios of 25:1, 5:1 and 1:1.

Discussion

We studied the ability of HLA-Ig-based aAPCs to stimulate clinically relevant CTL populations and modeled those studies on DC-mediated CTL expansion. This allowed us to compare the efficacies of aAPC-based and DC-based induction and expansion of antigen-specific CTLs. aAPC-based induction was at least as good as, if not better than, DC-based induction in all but one experiment. The ease of preparation of aAPCs and their stability also permit the inclusion of additional rounds of aAPC-mediated CTL expansion if desired. We maintained CTLs using aAPCs for more than two-and-a-half months of continuous culture, without any detectable loss in specificity or growth rate. Thus, HLA-Ig-based aAPCs represent a robust versatile technology useful for inducing and expanding antigen-specific cells.

For clinical studies, HLA-Ig–based aAPCs have several distinct advantages over cellular APCs, including DCs. aAPCs can be pre-

pared and stored for long-term use without loss of activity. aAPCs as described here are potentially relevant to all HLA-A2-restricted responses and can be easily adapted using other HLA-Ig complexes for other HLA-specific responses. Sterile cell culture and cytokines are not required to generate the aAPCs, reducing both the variability and expense associated with *ex vivo* expansion. The issue of variability is particularly important when considering therapies for cancer where defects in DCs obtained from patients have been reported[14]. The time-saving associated with an 'off-the-shelf' product can also be substantial in the setting of progressive disease.

An alternate approach has been to isolate antigen-specific cells and expand them on CD3-CD28 antibody beads[4]. Although this approach has proven useful for expansion of CD4+ cells, it produces only limited expansion of CD8+ cells[15]. This prompted the development of an artificial cell-based APC that expresses 4-1BB ligand (4-1BBL), to overcome the limited expansion seen with CD3-CD28 antibody beads[6]. The maintenance of antigenic specificity remains an issue with all CD3-specific antibody–based approaches, however, even when the starting populations are enriched for antigen-specific CTLs[6]. Using HLA-Ig–based aAPCs, we did not observe limited expansion or loss of specificity even after two months of continuous culture. Although CTL growth was not a limitation, we could have further augmented growth by including natural co-stimulatory ligands such as B7 (ref. 16), or other co-stimulatory molecules such as 4-1BBL and intercellular adhesion molecule-1 that minimize apoptosis[6,17]. Thus, the ability to deliver therapeutically active T cells is greatly augmented using HLA-Ig–based aAPCs.

The fact that we were able to expand Mart-1–specific cells is very promising. Starting with only 10⁶ CD8+ T lymphocytes, HLA-Ig–based aAPCs induced and expanded Mart-1–specific cells to clinically relevant amounts of CTLs (10^9 antigen-specific CTLs) in less than two months. Should larger numbers of antigen-specific CTLs be desired, this can be easily accomplished using additional rounds of aAPC-mediated stimulation. Alternatively, by starting with a single leukopack (500 ml of blood), one should be able to

Fig. 4 Frequency of antigen-specific CTLs after expansion with CD3-specific antibody beads or aAPCs. ***a–c***, T cells were stimulated with autologous monocyte-derived DCs pulsed with CMV peptide (*a*) to induce antigen-specific T-cell expansion. After 3 weeks of induction, T-cell populations were expanded on either CD3-CD28 antibody beads (*b*) or peptide-loaded HLA-Ig–based aAPCs (*c*). Cells were stained with FITC-conjugated CD8-specific monoclonal antibody and CMV peptide–pulsed A2-Ig loaded with pp65 (top panels) or with A2-Ig loaded with Mart-1 control peptide (lower panels). The percentage of peptide-specific CD8+ CTLs is shown in the upper right corner.
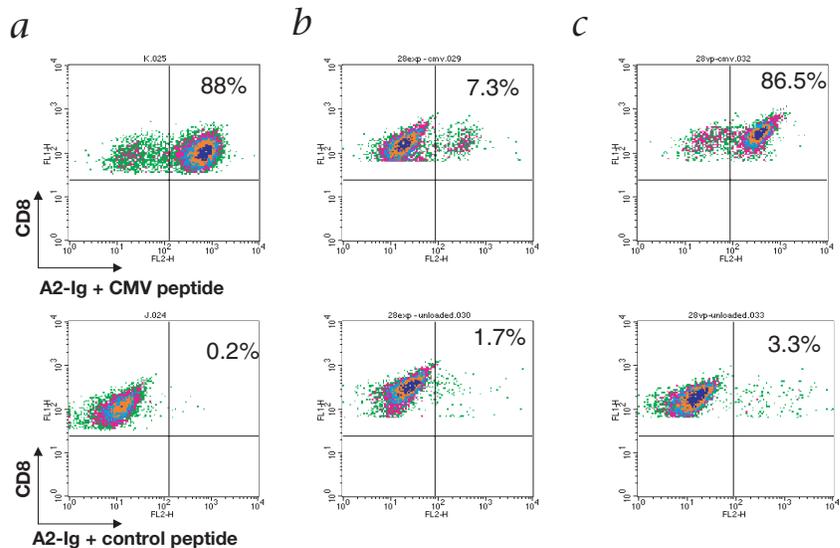
Methods

Cell lines. TAP (transporter associated with antigen processing)-deficient 174CEM.T2 (T2) cells and melanoma cell lines were maintained in M′ medium[18] supplemented with 10% fetal calf serum.

Peptides. Peptides used in this study (Mart-1, ELAGIGILTV, CMV pp65 and NLVPMVATV) were prepared by the Johns Hopkins University core facility. The purity (>98%) of each peptide was confirmed by mass-spectral analysis and high-pressure liquid chromatography.

HLA-A2.1[+] lymphocytes. The Institutional Ethics Committee approved this study. All donors gave written informed consent before enrolling in the study. Healthy volunteers and a melanoma patient (donor no. 7) were HLA-A2.1-phenotyped by flow cytometry. The melanoma patient had extensive metastatic disease with lung, liver and lymph-node metastases. PBMCs were isolated by Ficoll-Hypaque (Amersham Pharmacia Biotek, Uppsala, Sweden) density gradient centrifugation.

Generation of aAPCs. aAPCs were generated by coupling HLA-Ig and CD28-specific antibody onto microbeads (Dynal, Lake Success, New York). Briefly, beads were washed twice in sterile 0.1 M borate buffer. The beads were incubated with a 1-to-1 mixture of HLA-A2-Ig and the CD28-specific monoclonal antibody 9.3 in borate buffer for 24 h at 4 °C on a rotator, washed twice with bead-wash buffer, incubated again for 24 h at 4 °C in bead-wash buffer, and placed in fresh buffer. The resulting aAPCs were found to have 0.9×10^5 molecules of A2-Ig and 1.9×10^5 CD28-specific antibody molecules per bead. aAPC beads were stored at 4 °C for more than 3 months with no loss in activity. For peptide loading, HLA-Ig–coated aAPCs were washed twice with PBS and adjusted to 10^7 beads/ml in a final peptide concentration of 30 µg/ml. aAPC beads were stored in the peptide solution at 4 °C.

In vitro generation of dendritic cells. Monocytes were isolated from PBMCs by CD14^{+} magnetic separation (Miltenyi, Auburn, California). The CD14^{+} cells were cultured in M′ medium with 2% autologous serum, 100 ng/ml human granulocyte-macrophage colony-stimulating factor, 50 ng/ml IL-4, and 5 ng/ml transforming growth factor-β1. After 5–7 d of culture, a maturation cocktail containing 10 ng/ml tumor necrosis factor-α and IL-1β, 1,000 U/ml IL-6 (BD-Pharmingen, San Diego, California) and 1 mg/ml prostaglandin E2 (Sigma, St. Louis, Missouri) was added for 24 h. Cells displayed typical cell-surface markers of DCs (CD1a^{+}, CD14^{−} and CD86^{+}). For peptide loading, DCs were harvested and incubated with 30 µg/ml in M′ medium at a density of $1–2 \times 10^6$ cells/ml.

In vitro CTL induction. CD8^{+} T lymphocytes were enriched from PBMCs by depletion of CD8^{−} cells using a CD8 isolation kit (Miltenyi). The resulting population, consisting of >90% CD8^{+} T cells, was used as responder cells and stimulated with either peptide-pulsed DCs or peptide-pulsed aAPCs. Responder cells (10^4 cells/well) were then co-cultured with either 5×10^3 DCs or 10^4 peptide-pulsed aAPCs per well in a 96-well round-bottom plate in 200 µl/well M′ medium supplemented with 5% autologous plasma and 3% TCGF (T-cell growth factor). No additional allogeneic feeder cells were used for induction or expansion of CTLs. TCGF was prepared as described[9]. Medium and TCGF were replenished twice a week. On day 7 and weekly thereafter, T cells were collected, counted and re-plated at 10^4 T cells per well, together with either 5×10^3 peptide-pulsed autologous DCs or 10^4 peptide-pulsed aAPCs per well in complete medium supplemented with 3% TCGF.

Dimer and intracellular cytokine staining analysis. Cells were stained with FITC-conjugated CD8-specific monoclonal antibody and Mart-1– or CMV-pulsed A2-Ig, then conjugated with phycoerythrin-conjugated secondary antibody against mouse immunoglobulin to detect the dimer, as previously described[7]. For the control staining, A2-Ig was either loaded with an irrelevant peptide or unloaded, as indicated. Similar background staining was observed using either unloaded A2-Ig (as in Fig. 2) or A2-Ig loaded with an irrelevant A2-binding peptide (as in Fig. 4). For analysis, we gated on CD8^{+} cells.

Intracellular cytokine staining was performed as described (BD Pharmingen protocol) with the following modifications. Effector cells (10^6) were stimulated for 5 h at 37 °C with 2×10^5 peptide-pulsed T2 cells (30 µg/ml) or 10^6 melanoma cells. When melanoma cells were used as targets, 0.5 ng/ml phorbol 12-myristate 13-acetate (PMA) and 4 ng/ml ionomycin were added. Control experiments indicated that low doses of PMA and ionomycin did not induce cytokine production in the effector cells (data not shown). As previously described[19], a low dose of PMA and ionomycin increased the sensitivity of the intracellular cytokine staining assay. Differences in the results with or without this additional stimulation were dependent on the stimulus. The enhancement seen with low-dose PMA and ionomycin was more prominent when allogenic tumor cells were used as stimulator cells, up to 3–4-fold, but was insignificant when peptide-pulsed T2 cells or A293 cells were used to stimulate the antigen-specific T cells. The addition of low-dose PMA and ionomycin did not change background activity. The impact on in vivo experiments of using this approach to estimate antigen-specific CTLs in vitro, is not clear. The classic ^{51}Cr-release lysis assays were done without addition of PMA or ionomycin. Intracellular cytokine staining was often up to twice as sensitive as dimer staining, possibly because of heterogeneity in the induced CTL population[19]. Intracellular cytokine staining may detect a broader population of high- and low-affinity CTLs than dimers because of heterogeneity in peptide-induced antigen-specific CTL populations as previously reported[20]. The diversity in the repertoire may contribute to recognition by one but not by the other assay. Intracellular staining was done with FITC-labeled monoclonal antibodies against IFN-γ or IL-4 (BD Pharmingen). We found that a portion of the antigen-specific CTLs made either or both IFN-γ and IL-4, whether induced by aAPCs or DCs (see Supplementary Fig. 1 online). DC-based ex vivo expansion of human CD8^{+} cells making both IFN-γ and IL-4 has been observed[18]. Our results with aAPCs confirm these DC-based findings and show that aAPC-mediated stimulation results in phenotypically similar antigen-specific CTLs.

^{51}Cr-release assay. ^{51}Cr-release assays were performed as described[9]. CTL activity was calculated as the percentage of specific ^{51}Cr release using the following equation: percent specific killing = (sample release − spontaneous release) ÷ (maximal release − spontaneous release) × 100.

use aAPCs to induce and expand PBMCs to obtain 10^{11} CTLs in under two months.

Here, we showed that HLA-Ig–based aAPCs supported expansion of both a high-affinity antiviral CMV-specific CTL and a low-affinity Mart-1–specific CTL. Both specificities were expanded using the same basic aAPC protocol and aAPC-to-CTL ratio. One might have expected that induction and expansion of two such different CTL populations would have different stimulation requirements, but this was not the case. High-affinity interactions are known to induce significant apoptosis, yet expansion of CMV-specific CTLs was seen. This highlights the strength and versatility of the HLA-Ig–based aAPCs for clinical use. Considering the wide range of diseases that would be amenable to treatment given a robust, reproducible approach to *ex vivo* expansion of antigen-specific CTLs, HLA-Ig–based aAPCs could significantly advance the field of adoptive immunotherapy.

Note: Supplementary information is available on the Nature Medicine *website.*

Competing interests statement
The authors declare competing financial interests: see the Nature Medicine *website for details.*

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