Targeting human B-cell malignancies through Ig light chain specific cytotoxic T lymphocytes

Jinsheng Weng[1,3], **Soung-Chul Cha**[1,3], **Satoko Matsueda**[4], **Gheath Alatrash**[2,3], **Michael S. Popescu**[1,3], **Qing Yi**[1,3], **Jeffrey J Molldrem**[2,3], **Michael Wang**[1], **Sattva S. Neelapu**[1,3], and **Larry W. Kwak**[1,3]

[1]Department of Lymphoma and Myeloma, M.D. Anderson Cancer Center, Houston, TX

[2]Stem Cell Transplantation and Cellular Therapy, M.D. Anderson Cancer Center, Houston, TX

[3]Center for Cancer Immunology Research, M.D. Anderson Cancer Center, Houston, TX

[4]Department of Immunology and Immunotherapy, Kurume University School of Medicine, Japan

Abstract

Purpose—The variable regions of Ig (idiotype, Id) expressed by malignant B cells can be used as tumor-specific antigens that induce humoral and cellular immunity. However, epitopes derived from Id that stimulate human CD8+ T-cell immunity are incompletely characterized.

Experimental design—The clonal Ig V_L of human myeloma cell line U266 and five primary B-cell tumors were sequenced and peptides corresponding to the Ig V_L region were tested for their ability to stimulate cytotoxic T lymphocytes (CTLs) from ten HLA-A* 0201 positive normal donors. The CTLs thus generated were tested against peptide-pulsed T2 cells and autologous tumor cells.

Results—14 peptides derived from Ig light chain (V_L) of U266 and primary B-cell tumors were used to generate 68 Cytotoxic T lymphocytes (CTLs) lines that specifically produced IFN-γ when co-cultured with peptide-pulsed T2 cells. These CTLs lysed peptide-pulsed T2 cell as well as U266 or autologous tumor targets in an HLA class I-dependent manner. Sequence analysis revealed shared V_L T-cell epitopes in U266 and primary B-cell tumors, not previously reported within Ig heavy chain (V_H) sequences.

Conclusion—This study thus identifies novel immunogenic CTLs epitopes from Id V_L, suggests that they are naturally presented on the surface of B-cell malignancies, and supports their inclusion in next generation Id vaccines. The ability to prime T cells derived from normal HLA-matched donors, rather than patients, also may have direct application to current strategies, designed to generate allogeneic tumor-specific T cells for adoptive transfer.

Keywords

Address correspondence to Larry W. Kwak, M.D., Ph.D., Professor and Chairman, Department of Lymphoma and Myeloma, The University of Texas M. D. Anderson Cancer Center, 1414 Holcombe Boulevard, Unit 429, Houston, TX 77030, Phone: 713-745-4252, Fax: 713-563-3424, lkwak@mdanderson.org.

Introduction

B-cell malignancies express unique variable region determinants in their surface Ig receptor (Id) that can serve as tumor-specific antigens. Studies in mice and humans showed that humoral and cellular immune responses were induced following Id vaccination (1–4). We have previously demonstrated that autologous Id protein can be formulated into an immunogenic antigen in lymphoma patients, by conjugation with a carrier protein, keyhole limpet hemocyanin (KLH), and administration with GM-CSF as adjuvant. Lymphoma-specific CD8+ T-cell responses were associated with achievement of molecular remissions (3). In human myeloma patients, T-cell responses specific for Id protein have generally been demonstrated, suggesting immunogenicity of this tumor antigen (5). Finally, a randomized Phase III clinical trial of an Id protein vaccine recently demonstrated prolonged remission duration in follicular lymphoma (FL) patients in first remission(6). However, the immunogenic epitopes derived from Id that stimulate CD8+ T-cell responses have been incompletely characterized, especially Id light chain (V_L) determinants.

Despite the availability of new proteosome inhibitors and other targeted agents, disease relapse still remains a major problem for myeloma patients, and even high dose therapy followed by autologous stem cell transplantation (SCT) in tandem does not appear to be curative for this disease (7). In contrast, allogeneic SCT following either myeloablative or reduced-intensity conditioning has been shown to induce prolonged disease-free survival in a small percentage of patients suggesting a possible graft versus myeloma (GVM) effect(8). Attempts to enhance the GVM effect by donor lymphocyte infusions (DLI) have resulted in an increased incidence of graft versus host disease (GVHD)(9). Therefore, strategies to enhance the specific antitumor effect of the graft without increasing the risk of GVHD are needed to improve outcome in allotransplant recipients.

One novel strategy is to transfer highly-enriched populations of tumor antigen-specific T cells from donor to recipient (i.e., educated donor lymphocyte infusions, DLI) to enhance the antitumor effect of the allograft without exacerbating GVHD. The approach of allogeneic marrow donor immunization in myeloma has been tested clinically in a small number of HLA-matched donor-recipient pairs and donor immunization with Id protein has proved safe (10, 11). As an alternative to vaccinating donors in vivo in future clinical studies, we develop here a method to prime and expand donor idiotype light chain-specific T cells in vitro with the goal of using Id-specific DLI as the transfer element against B-cell malignancies in future clinical studies.

Materials and Methods

Human tumors

U266 myeloma cell line (HLA-A*0201/A3+) was obtained from ATCC. HLA-A*0201 primary FL or chronic lymphocyte leukemia (CLL) tumors were purified from patient's blood or spleen with HISTOPAQUE-1077 (Sigma) and B-cell isolation kit (Miltenyi Biotec). HLA-A*0201 primary plasma cell leukemia cells (PL) were isolated with CD138+ cell isolation kit (Miltenyi Biotec). All patients' samples were collected before the administration of high does therapy or idiotype vaccination. This study was approved by the Institutional Review Board Committee and informed consent was obtained in accordance with the Declaration of Helsinki.

RT-PCR of idiotype light chain cDNA

3μg RNA extracted from U266, primary tumors was reverse- transcribed into cDNA with Superscript III kit from Invitrogen (cat# 11745100). The highly variable region of idiotype light chain region was PCR amplified with primers from published paper (12). The PCR

conditions are: 94°C 5min, followed by 94°C, 30sec, 58°C, 30sec, 72°C, 45sec for 35 cycles, 72°C, 9 minute. The PCR product was cloned into PCR2.1 TOPO vector (Invitrogen, cat #K2000-01) and sequenced in the DNA core facility of MD.Anderson Cancer Center.

Peptide synthesis and T2 binding

Peptides predicted to bind to HLA-A*0201 were synthesized to greater than 70% purity, dissolved in 100% dimethyl sulfoxide (Sigma), and the binding affinity to HLA-A*0201 molecules was measured with T2 cells according to published methods (13). In brief, T2 cells were incubated with 50µg/ml peptides and 3 µg/ml of β_2-microglobulin (Sigma, cat # M4890) for overnight. The cells were then washed and incubated with PE-labeled anti–HLA-A0201 mAb (clone BB7.2, BD Biosciences) for 30 minutes at 4°C. After washes and fixation, cells were analyzed for the levels of HLA-A2 expression by flow cytometer. The binding affinity of peptide was quantified according to the formula [(mean fluorescence with peptide − mean fluorescence without peptide)/mean fluorescence without peptide] × 100%. Influenza A virus M1 58–66 (GILGFVFTL) peptide was used as a positive control.

Generation of peptide-specific CTLs

CTLs were generated from HLA-A*0201 normal donor PBMCs using reported methods (14). Briefly, PBMCs (1×10^5 cells/well) were incubated with 10 µg/ml peptide in quadruplicate in 96-well U-bottom-microculture plates in 200 µl culture medium (50% AIM-V, 50% RPMI-1640, 10% human AB serum, 100 IU/ml of IL-2) and restimulated with peptide every 3 days. After 5 stimulations, T cells were stimulated with peptide-pulsed T2 cells and interferon (IFN)-γ production was determined in supernatants by ELISA. CD8+ T cells were isolated from IFN-γ-producing cultures by MACS and expanded by rapid expansion protocol(15). Influenza A virus M1 58–66 (GILGFVFTL) peptide was used as a positive control.

Cytotoxicity

U266 and primary tumor cells were labeled with ^{51}Cr and standard 4-hour cytotoxicity assay was performed. Anti-human HLA-ABC (clone W6/32, eBiosciences), anti- HLA-DR, DP, DQ (clone TÜ39, BD Biosciences), and Mouse IgG2a Isotype Control (eBiosciences, cat# 16-4724-81) were used to determine HLA restriction. All assays were performed in triplicate and repeated three times.

Intracellular cytokine staining assay

Effector T cells were mixed with T2 or antigen presenting cells (APCs) loaded with 10µg/ml peptide at 1: 1 ratio. 2 hour later, 5µg/ml Brefeldin A (Sigma) was added to block the transfer of Gogi part. The staining of intracellular cytokine was performed with BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization kit 12 hour later. 10µl mouse anti-human IFN-γ (clone 25723.11, BD Biosciences), mouse anti-human TNF-a (clone MAb11 BD Biosciences), mouse anti-human GM-CSF(clone 4H1, eBiosciences), mouse anti-human IL-4 (clone 8D4,BD Biosciences), mouse anti-human IL-10 (clone: JES3-9D7, eBiosciences), mouse anti-human IL-17 (clone eBio64DEC17, eBiosciences), mouse anti-human CD8 (clone HIT8a, BD Biosciences) were added to the 100µl effector T cells and stained for 30minutes at 4°C in the dark. After 2 times washes in 1×Perm buffer, the samples were analyzed by flow cytometer and the data was analyzed with cell Quest software or Flowjo.

Statistical analysis

The Student t test was used to compare various experimental groups. Unless otherwise indicated, the mean average and standard deviations (SD) of triplicate wells are shown.

Results

Generation of Id V$_L$-specific T-cell lines from normal donors

As a model, we used peptides derived from the V$_L$ of human U266 myeloma cell line to generate CTLs. Eighteen peptides from lambda V$_L$ of U266 were selected based on the predicted binding affinity to HLA-A*0201 (http://www-bimas.cit.nih.gov/molbio/hla_bind/). Most peptides showed modest binding capacity to HLA-A*0201 molecules (T2 binding index: 0.15–1.29) (Supplementary Table 1). As peptides of low affinity are capable of inducing CTLs (16), all were used to generate CTLs from HLA-A* 0201 normal donors (14). Nine U266 peptides (P19, P20, P21, P23, P25, P26, P27, P28, and P29) induced CTLs that specifically secreted IFN-γ when co-cultured with peptide-pulsed T2 cells (Figure 1A). These peptides induced 54 CTLs in ten HLA-A* 0201 donors (Table.2), confirming their immunogenicity in general normal donors. Using intracellular cytokine assay, we demonstrated that CTLs produced IFN-γ, TNF-α, and GM-CSF but not IL-4, IL-10, or IL-17 cytokines (Figure.1C). Next, we used 105 candidate peptides from V$_L$ of CLL (n=1), FL (n=3), and PL (n=1) tumor cells and demonstrated that five primary Id V$_L$-derived peptides are also immunogenic to generate 14 CTLs from ten normal donors (Figure 1B and Table.2).

Cytotoxicity of V$_L$ peptide-specific donor T cells

We expanded peptide-specific CTLs and assayed for cytotoxicity against peptide-pulsed T2 cells and primary tumors. Both U266 peptide-specific CTLs (Figure 2A) and CTLs generated against V$_L$ peptides from primary tumors (Figure 2B) efficiently lysed Id, but not control peptide-pulsed, T2 targets.

Peptide-specific CTLs raised against multiple U266 peptides also lysed U266 as target efficiently. CTLs did not lyse myeloma targets expressing irrelevant Id (ARP-1, HLA-A2−/Id-; XG1, HLA-A2+/Id-), indicating their specificity (Figure 3A). Donor-derived CTLs raised against primary tumor-derived V$_L$ peptides also specifically lysed primary tumors but not tumors expressing irrelevant Ids, or the respective patients' PBMC (Figure 3B) or K562 cells (not shown).

To further characterize effector T cells, we assayed for inhibition of tumor cell lysis by HLA-blocking antibodies. We observed that HLA class I, but not class II or isotype control antibodies inhibited cytolysis by U266 peptide-specific CTL lines (Figure 4A). HLA class I antibodies also inhibited the lysis of primary tumor cells by CTL raised against their respective V$_L$ peptides, suggesting that V$_L$ peptides were processed and presented on HLA class I molecules by both U266 and primary B-cell tumors (Figure 4B).

Candidate V$_L$ epitopes shared by human FL

A current limitation of Id vaccines is the requirement for individualized manufacture which could be partially overcome by identification of universal T-cell epitopes shared by multiple patients. To determine whether the immunogenic U266 and primary tumor V$_L$ peptides were shared by other human B-cell tumors, we compared them to 90 lambda and 123 kappa L-chain sequences from a panel of FL tumors (D. Gold, unpublished data). Interestingly, we observed that 12 FL tumors shared the P21 sequence while one FL each shared P19 and P25 epitopes. Seven FL shared the L50 epitope, two FL each shared the L53, L54 and L61 epitopes, and K18 and L10 epitope were shared by one other primary FL each (Table 1). The two most frequently shared peptides (P21 and L50) localized to the framework regions of V$_L$ and consisted of germ line sequences, without somatic mutation. Overall, 27 out of 90 (30%) FL tumors expressing lambda L-chain shared at least one of the 13 epitopes we

identified. Analysis of shared kappa epitopes will require additional studies, as only a single immunogenic kappa epitope was identified here.

To determine whether CTLs generated against a U266 peptide could lyse primary tumor cells expressing a shared epitope, we incubated donor-derived, P21-specific CTLs with HLA-A*0201 FL that expressed the P21 epitope (FL4, HLA-A2+/P21+). We observed significant lysis of FL target cells, but not PBMC from this patient or FL that did not express P21 (FL5,HLA-A2+/P21−), suggesting specificity for Id (Figure 4C).

Discussion

Increasing data suggest that human T-cell immunity plays an important role in anti-tumor effects induced by Id vaccination with whole protein (17–19). Studies have identified T-cell epitopes harbored by Ig V_H of human and Ig V_L of murine B-cell tumors (20–29), but whether human Id V_L harbor important T cell epitope remains unclear(25, 26). Two previous publications reported that Ig light chains contained few T cell epitopes. In Dabadghao's study, PBMC from five myeloma patients were incubated with fragment F(ab)2, Fab, HC (heavy chain) or LC (light chain) of autologous idiotype protein to determine idiotype specific T cell proliferation. They found idiotype fragments of F(ab)2, Fab, HC can stimulate PBMC proliferation. However, none of the patients' PBMC proliferated to LC of idiotype protein (25). In the study by Fagerberg et al., peptides corresponding to the heavy chain and light chain of one myeloma idiotype protein were synthesized and incubated with one myeloma patient's PBMC. Using ELISPOT assay, they found only peptides derived from HC but none from LC of idiotype protein could stimulate T cells to secret IFN-γ (26). In our study, we have identified 14 peptides from Ig light chain (V_L) of U266 and primary B-cell tumors that can be used to generate 68 CTLs lines in vitro. These V_L CTLs lysed the peptide pulsed-T2 cell as well as tumor cells, indicating that V_L immunogenic epitopes are processed and presented by tumor cells. The differences between our results and previous studies may have resulted from several reasons. First, the patient's PBMC is not ideal to study protective T cell immunity, as the immune balance is skewed towards immune suppression (30). Second, the whole idiotype protein, may harbor immune regulatory, as well as stimulatory sequences, thus the identification of immunogenic epitope may be hampered by regulatory epitopes on the whole idiotype protein(31) Lastly, significantly more peptides from patients were synthesized and tested in our study. Overall, our study suggests that immunogenic epitopes are present in V_L CDR and FWR regions, are processed and presented by primary tumor cells, and supports strategies for targeting V_L epitopes on B-cell tumors. CTLs targeting Id V_L may also have specific clinical usage against L-chain-only secreting plasma cell dyscrasias (27).

Id vaccination significantly extended the disease-free survival of follicular lymphoma patients as demonstrated in a recent controlled Phase III trial, consistent with suggestions from prior pilot clinical trials (6, 32). The ability to prime HLA-A2 + donor CD8+ T cells against Ig V_L epitopes may provide a strategy for improving adoptive therapy against B-cell malignancies. Previous work in human SCT, demonstrating that the transfer of humoral, and to a lesser extent cellular, antigen-specific immunity to clinically important viral antigens from immune donors to recipients can occur, provides a rationale for transferring tumor antigen-specific immunity induced in donors(33–35). Vaccination of HLA-matched sibling donors with myeloma Id protein, with subsequent transfer of Id-specific immunity by stem cell transplantation, has been shown to be feasible in a limited number of patients with myeloma (10, 11). Six donors were vaccinated with Id proteins (conjugated to KLH) isolated from the plasma of the myeloma patients prior to marrow harvest, and respective recipients were administered booster Id immunizations following transplantation. The vaccine was well-tolerated by all donors and recipients. With median follow-up of eight

years, no long-term toxicity has been observed in any immunized donor. Vaccination induced specific cellular and/or humoral immune responses against Id and the vaccine carrier, KLH, in all donors. Two patients died within 30 days of SCT due to transplant-related complications, but Id-specific and KLH-specific T-cell responses were detected in all four remaining patients post-, but not pre-BMT. All four surviving patients converted from partial to complete responses following SCT. Taken together, these preliminary results suggest that 1) idiotype protein can elicit a specific immune response in a healthy donor, 2) direct transfer of idiotype specific T-cell immunity can occur from donor to recipient, and 3) donor-derived T-cell responses may not be blocked by circulating idiotype protein present in the patients during and after SCT, or by iatrogenic immunosuppression for GVHD prophylaxis. Our current data suggest that as an alternative to donor vaccination, immunogenic peptides may be used to selectively expand Id-specific T cells ex vivo to generate "educated" donor lymphocyte infusions (DLI). Such primed T cells might enhance tumor specificity and limit graft-vs.-host disease complications of current DLI strategies.

Using a recently described method (14), we completed multiple T-cell stimulations within 2 weeks, such that the T cells are not exhausted at that time. Instead, these T cells are at much younger stage compared to T cells traditionally stimulated 3 to 4 times weekly. Even after these multiple stimulations, we observed that the T cells retained effector function and were able to lyse peptide-pulsed and tumor cell targets (Figures 2–3). Furthermore, as described, using an established rapid expansion protocol, we demonstrated feasibility of expanding such idiotype-specific T cells in large numbers. We are thus now in a position to perform future in vivo experiments to determine whether adoptively transferred T cells recognizing shared epitopes would persist and be active.

Finally, although outside of the scope of the current study, one of the important questions in human tumor immunology is to determine whether autologous tumor-specific CTLs pre-exist in patients. In light of the immunogenicity of idiotype light chain peptides found in this study, specifically, it will be important to determine whether Ig light chain specific CTLs pre-exist in the blood of patients with B cell tumors and whether such precursors can be expanded by vaccination or adoptive transfer. Previous studies have suggested the existence and function of such tumor specific T cells in B cell tumor patients (36, 37). Experiments testing the ability of human tumor V_L peptides to generate autologous Id-specific T cells are under active investigation in our laboratory.

Translational Relevance

Despite improvements in high dose therapy followed by autologous stem cell transplantation (SCT), disease relapse still remains a major problem for B cell malignancy patients, and even tandem autografts do not appear to be curative for these diseases. Allogeneic SCT following either myeloablative or reduced-intensity conditioning has been shown to induce prolonged disease-free survival in a small percentage of patients suggesting a possible graft versus myeloma (GVM) effect. The ability to prime tumor-specific T cells derived from normal HLA-matched donors, rather than autologous patients, may have direct application to current adoptive transfer strategies, already in clinical testing.

Acknowledgments

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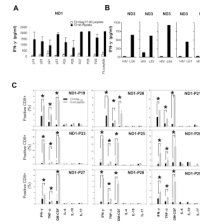
Figure 1.
Generation of Id V_L peptide-specific T cells from 10 normal donors (ND1–10). (A) IFN-γ secretion by U266 myeloma cell line V_L peptide-specific CTLs after stimulation with peptide-pulsed T2 cells or HIV-Gag77 peptide-pulsed T2 cells (negative control). Influenza A virus M1(58–66) peptide was used as a positive control. Id V_L peptide sequences and characteristics are shown in Table.1 (B) IFN-γ secretion by primary B-cell tumor-derived V_L peptide specific CTLs against peptide pulsed or HIV-Gag77 peptide-pulsed T2 cells. (C) Intracellular cytokine staining assay of peptide-specific CTLs in response to V_L peptide or HIV-Gag77 peptide-pulsed T2 cells. * indicates $P<0.05$.
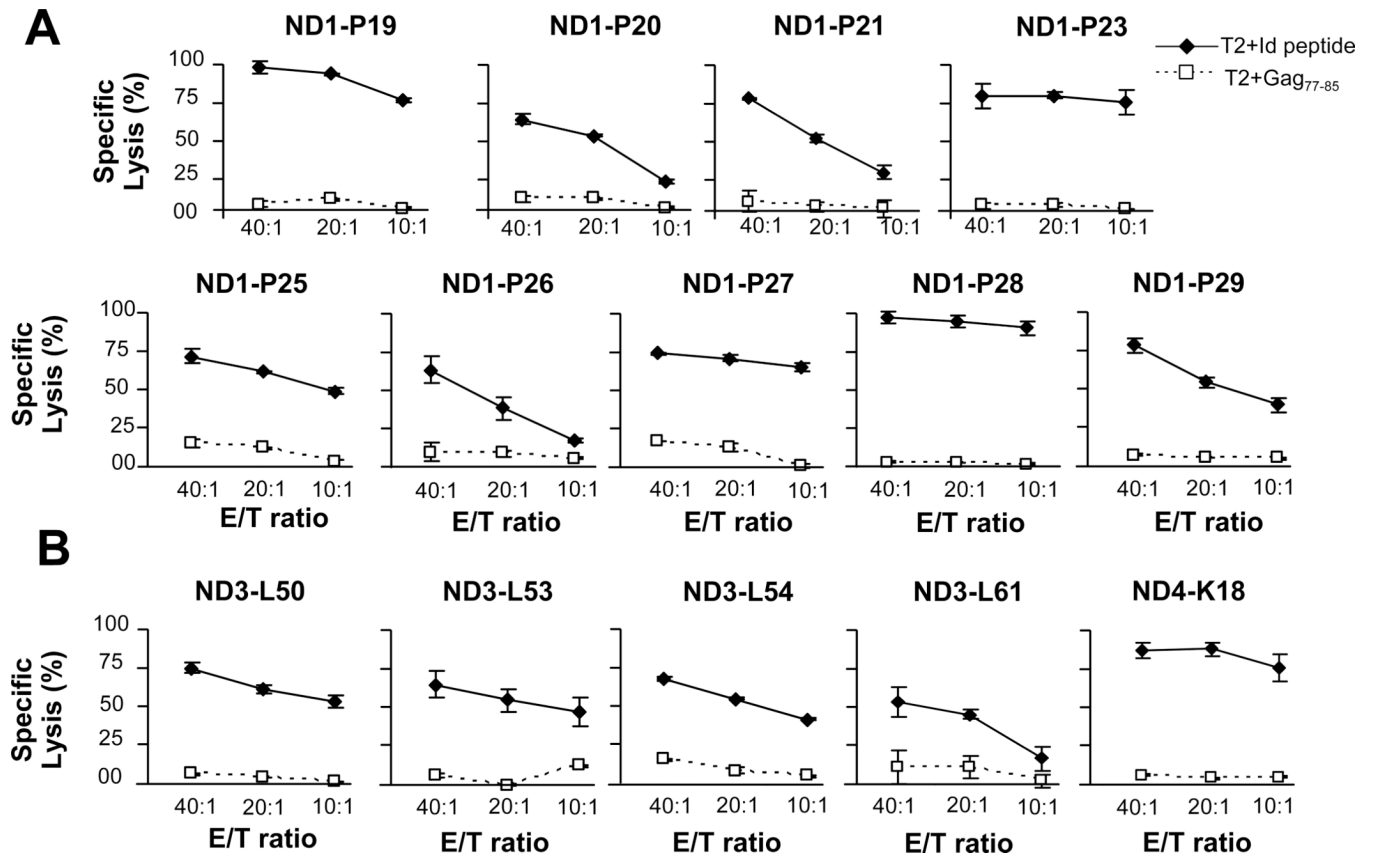
Figure 2.
The cytotoxic function of V_L peptide-specific CTLs against Id peptide or control HIV peptide pulsed T2 cells. (A)The cytotoxic function of U266 derived V_L peptide-specific CTLs was tested against T2 cells pulsed with U266 or control HIV peptide. (B) The cytotoxic function of donor CTL raised against primary B-cell tumor-derived V_L peptides was tested against T2 cells pulsed with the respective human tumor-derived or control HIV peptide. Results are representative of three independent experiments.
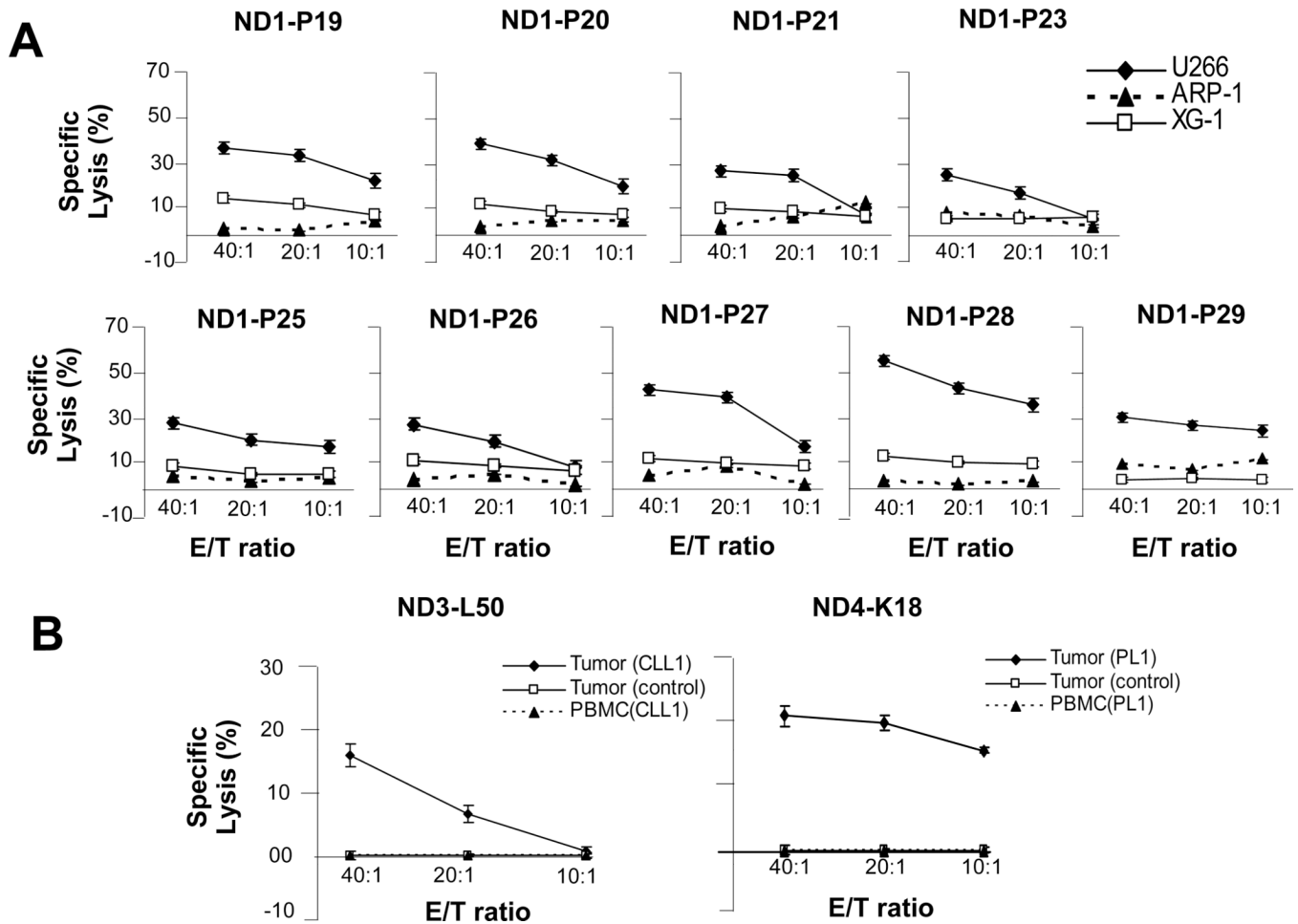
Figure 3.
Cytotoxic function of V_L peptide-specific normal donor CTLs against U266 and primary human B-cell tumor targets. (A) U266 V_L peptide-specific (P19, P20, P21, P23, P25, P26, P27, P28, P29) CTLs generated from a normal donor (ND1) lysed U266 myeloma cells but not ARP-1 (HLA A2−, irrelevant Id) or XG-1 (HLA A2+, irrelevant Id) myeloma cell lines. (B) Human tumor-derived V_L peptide-specific (L50 and K18) CTLs lysed the respective primary tumor cells: Tumor (CLL1) and Tumor (PL1), but not tumors expressing irrelevant Id: Tumor (control), or autologous patient's tumor-free PBMC: PBMC(CLL1) and PBMC(PL1).
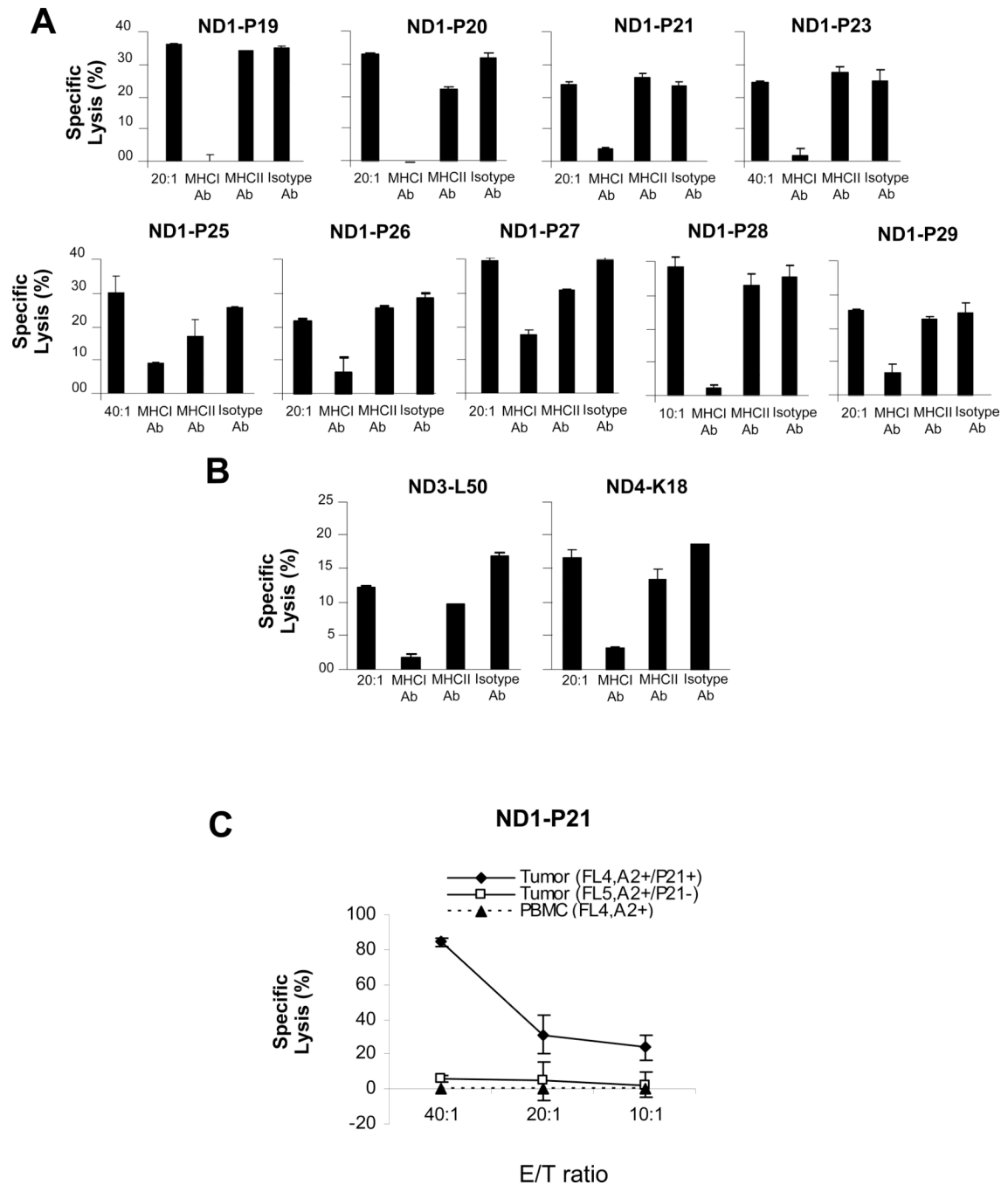
Figure 4.
HLA restriction of of V_L peptide specific CTLs. (A) HLA class I but not HLA class II specific antibody or isotype control antibody (10 µg/ml) blocked the cytolysis of U266 tumor cells by U266 V_L peptide-specific CTLs. (B) HLA class I but not HLA class II antibody or isotype control antibody (10 µg/ml) blocked the cytolysis of primary human CLL and PL tumor cells, respectively by V_L peptide-specific CTLs. (C) U266 P21 peptide-specific CTLs specifically lysed HLA-A*0201+ primary FL cells that shared the P21 epitope: Tumor (FL4, P21+), but not the same patients' PBMC: PBMC(FL4) or HLA-A*0201 tumor cells that did not express P21(FL5,P21−).

Table 1

Characteristics of V_L T-cell epitopes from U266 and primary B-cell tumors

Tumor source	Peptide	Variable Region[a]	Subfamily[b]	start position[c]	Sequence[d]	Length	Predictive binding[e]	Mean Fluorescence[f]	shared tumors[g]
	P19	FR1	IGLV1	5	TQPPSTSET	9	0.76	0.40	1/90
	P20	FR2	IGLV1	39	**HLPGKAPKL**	9	0.73	0.26	0/90
	P21	FR3	IGLV1	71	SASLAISGL	9	0.68	0.15	12/90
	P23	CDR1	IGLV1	26	TSNIGSNSV	9	0.45	0.24	0/90
U266 Myeloma	P25	CDR1	IGLV1	32	NSVNWY**QH**L	9	0.21	1.29	1/90
	P26	CDR3	IGLV1	90	ASWDDRLNGL	10	10.92	0.26	0/90
	P27	CDR1	IGLV1	9	STSETPGQGV	10	3.96	0.53	0/90
	P28	FR1	IGLV1	17	GVTISCSGST	10	0.09	0.15	0/90
	P29	FR1	IGLV1	11	**SET**PGQGVTI	10	0.20	0.45	0/90
Human CLL1	L50	FR1	IGLV2	29	SVSGSPGQSI	10	0.913	0.07	7/90
Human FL1	L53	FR1	IGLV1	28	SASATPGQRV	10	0.96	0.07	2/90
Human FL2	L54	FR1	IGLV4	29	SASASLGASV	10	0.96	0.06	2/90
Human FL3	L61	FR1	IGLV1	16	KVTISCSGST	10	0.29	0.06	2/90
Human PL1	K18	CDR2	IGKV3	50	LIYGTS**S**RA	9	3.7	0.52	1/123
								Lambda	27/90
								kappa	1/123

[a,b,c] Analysis and blast by IMGT website (http://imgt.cines.fr/IMGT_vquest/vquest/vquest?livret=0&Option=humanIg)

[d] Somatically mutated amino acids are bolded

[e] Estimate of half-time of disassociation and calculated score in arbitrary units.

[f] Mean fluorescence Index = (Mean fluorescence with peptide-mean fluorescence without peptide)/(mean fluorescence without peptide).

[g] Individual peptide sequences were compared with Ig V-region sequences available from either 90 FL patients with lambda or 123 patients with kappa L-chain isotype, respectively.

CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; PL, plasma cell leukemia

Table 2

Immunogenicity of Id V$_L$ peptides derived from human U266 myeloma cell line and primary B-cell tumors

Petides	Peptide specific IFN-γ (pg/ml) secretion[b]									
	ND1[a]	ND2	ND3	ND4	ND5	ND6	ND7	ND8	ND9	ND10
P19	1431.9	904.4	2039.7	1339.2	2095.9	0.0[c]	0.0	1245.6	1563.8	0.0
P20	1203.9	333.0	2208.1	0.0	1432.5	0.0	0.0	1023.5	0.0	0.0
P21	710.2	0.0	0.0	0.0	1028.7	0.0	0.0	4512.2	0.0	0.0
P23	1817.3	107.1	1976.4	1649.5	1382.4	1542.3	0.0	3215.3	0.0	1046.7
P25	1091.1	2075.4	2292.2	0.0	2035.1	2365.2	1423.2	3351.2	0.0	0.0
P26	716.0	1239.0	1303.5	1856.0	1241.9	2548.2	1122.5	0.0	0.0	0.0
P27	1931.2	117.4	2297.3	0.0	2248.5	0.0	0.0	2534.9	1333.5	0.0
P28	2126.6	1870.5	2071.9	1176.6	2640.2	0.0	0.0	3081.5	0.0	0.0
P29	1527.9	1186.7	2256.6	1785.8	2677.5	0.0	0.0	0.0	0.0	0.0
L50	0.0	0.0	605.0	0.0	585.0	487.3	0.0	569.8	0.0	0.0
L53	0.0	0.0	401.3	0.0	0.0	455.6	0.0	399.6	0.0	0.0
L54	0.0	0.0	863.5	0.0	578.2	0.0	0.0	0.0	601.3	0.0
L61	0.0	0.0	348.6	0.0	0.0	0.0	0.0	123.5	0.0	0.0
K18	0.0	0.0	0.0	598.4	0.0	0.0	0.0	232.5	0.0	0.0

[a] ND = normal donor

[b] Peptide specific IFN-γ secretion = IFN-γ from T cells stimulated with Id V$_L$ peptide pulsed T2 cells - IFN-γ from T cells stimulated with control peptide (HIV Gag77–85) pulsed T2 cells.

[c] no peptide specific IFN-γ secretion detected