

Identification of Human Idiotypic-Specific T Cells in Lymphoma and Myeloma

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Abstract Idiotypic protein, among the first identified tumor-specific antigens, has been found to stimulate both humoral and cellular responses in lymphoma and myeloma patients. With the increasing use of B cell depletion treatments such as rituximab in clinic, the cellular response mediated by idiotypic-specific T cells has become increasingly important as an adjunct therapy for lymphoma and myeloma. Here, we review the idiotypic protein as a tumor antigen and the characteristics of the T cell response elicited by idiotypic vaccination. We also analyze the T cell epitopes that have been identified in idiotypic protein and introduce our new findings of additional T cell epitopes derived from the Ig light chain. Finally, we propose new directions in the generation of idiotypic-specific T cells for tumor therapy.

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1 Introduction

Vaccination against infectious diseases has been a great success of modern medicine. Since the discovery that many human tumors contain tumor-specific antigens that can stimulate human immune response, there has been much effort to use this knowledge for immunotherapy during the past decades (Hareuveni et al. 1990). The fact that malignant B cell tumors such as lymphoma and myeloma produce a tumor-specific immunoglobulin, known as “idiotype” (Id) protein, has been known for more than 30 years (Sirisinha and Eisen 1971; Lynch et al. 1972). Early studies of idiotype protein immunotherapy were focused on humoral immune responses as anti-idiotype protein antibodies could easily be detected in vaccinated animals or patients (Stevenson and Stevenson 1975). With the development of new techniques in immunology, a cellular response has been frequently observed in idiotype protein-vaccinated patients, particularly with idiotype-specific T cells.

2 Idiotype Protein as a Tumor Antigen: Discovery Research

Idiotype refers to the very tip of the F(ab) region of immunoglobulin that mediates the binding of antibody with specific antigen. Immunoglobulin can either be expressed on the cell surface of lymphoma cell or secreted in the serum by myeloma cells. The cell surface immunoglobulin mediates important cell functions for B cells including antigen recognition, cell growth, and cell survival (Kuppers 2005; Herzog et al. 2009). Under normal conditions, each B cell synthesizes or secretes only one type of immunoglobulin that is unique to the B cell. Since lymphoma and myeloma are clonal B cell tumors, the immunoglobulin produced by lymphoma and myeloma has been considered a tumor-specific antigen (Kwak and Longo 1996). In addition, studies have shown that there are few lymphomas or myelomas that lose the expression of immunoglobulin (Kuppers 2005). Hence, the idiotype proteins of lymphoma or myeloma have several desirable characteristics of an ideal tumor antigen: (1) they are essential to cell function, (2) cell specific, (3) and over expressed in tumors, thus making them ideal candidates as targets for tumor immunotherapy.

The preclinical rationale for idiotype vaccination was first demonstrated by Lynch and Eisen in the 1970s and later confirmed by others in different tumor models (Lynch et al. 1972; Stevenson and Stevenson 1975; Kaminski et al. 1987). Kwak, working in the laboratory of Levy at Stanford University, pioneered the first study translating therapeutic idiotype vaccines to human patients (Kwak et al. 1992). This small pilot study demonstrated that specific antibody responses could be elicited in patients with low-grade lymphoma. Subsequently, the Kwak laboratory at the National Cancer Institute (NCI) discovered that the addition of GM-CSF substantially improved vaccine potency and successfully induced T cell responses in murine lymphoma models (Kwak et al. 1996).

3 Clinical Investigational New Drug Vaccine Development

With dedicated process development support by the Biological Resources Branch at NCI, the first investigational new drug (IND)-supported study of the prototype idiotype vaccine product (patient-specific, hybridoma-generated, idiotype protein chemically linked with keyhole limpet hemocyanin [KLH]) was a phase II clinical trial for previously untreated patients with follicular lymphoma (FL) by the Kwak laboratory (Bendandi et al. 1999). This trial used soluble GM-CSF as an integral part of the vaccine. All patients on this study received treatment with a uniform chemotherapy regimen, which produced a homogeneous group of 20 patients who were all in first complete remission (minimal residual disease state). After a 6-month break following induction therapy (to allow for immune reconstitution after the immunosuppressive effects of chemotherapy), soluble, recombinant GM-CSF was mixed with Id-KLH, and the complete vaccine was injected s.c. in 5 monthly doses. This study was the first to show convincing lymphoma-specific CD8⁺ T cell responses in the vast majority of patients (85%). In addition, evidence for molecular remissions was obtained in most of the subset of patients whose lymphomas could be detected by this technique. Particularly compelling were the clinical outcomes of this phase II study. After a median follow-up of 9.2 years, the median disease-free survival (DFS) was 8 years and overall survival was 95% (Bendandi et al. 1999). The DFS was superior to that of a historical, ProMACE chemotherapy-treated control group (median DFS, about 2.2 years) (Longo et al. 2000). These results were subsequently confirmed in other, non-IND studies of various vaccine formulations using hybridoma-generated idiotype proteins (Houot and Levy 2009).

4 Pivotal Phase III Trials

Three randomized double-blind placebo controlled multicenter clinical trials were designed to formally determine the clinical efficacy of therapeutic idiotype vaccination in FL. The first phase III trial was initiated by the NCI, and subsequently sponsored by Biovest International, Inc. Patients with previously untreated advanced stage FL were treated with PACE chemotherapy regimen until clinical remission. Patients achieving complete remission were randomized at a ratio of 2:1 to receive Id-KLH (BiovaxID) plus GM-CSF or KLH plus GM-CSF. The primary endpoint for this trial was DFS. About 177 out of 234 enrolled patients achieved complete response and were subsequently randomized to receive either active or control vaccine. Of these, 117 patients maintained remission for the 6-month rest period and received at least one dose of vaccine. This group of patients were treated as intended, and as such constituted the modified intent-to-treat analysis. Seventy-six patients received Id-KLH plus GM-CSF and 41 patients received KLH plus GM-CSF. Both arms were balanced

for International Prognostic Index (IPI) and other relevant clinical factors. After a median follow-up of 56.6 months (range 12.6–89.3 months), median time to relapse after randomization for the Id-KLH/GM-CSF arm was 44.2 months vs. 30.6 months for the control arm (P -value = 0.045; HR = 1.6) (Schuster et al. 2009).

The other two phase III trials differed in terms of induction therapy, eligibility of the patients, and method of idiotype production. The Genitope sponsored trial used CVP chemotherapy regimen (cyclophosphamide, vincristine, and prednisone), enrolled complete response and partial response patients, and used recombinant DNA technology for production of idiotype protein. Eligible patients were randomized to receive MyVax (Id-KLH+GM-CSF) or control vaccine (KLH+GM-CSF) in a 2:1 ratio for 6 months after completion of eight cycles of CVP. A series of seven immunizations was administered over 24 weeks. Out of 287 randomized patients 278 received at least 1 vaccination. Forty-one percent of evaluable patients showed specific anti-Id antibody responses. No statistical difference was observed between the two arms in terms of PFS (Levy et al. 2008). The third phase III trial, sponsored by Favril, investigated the efficacy of recombinant Id-KLH vaccination after treatment with rituximab. Previously untreated and relapsed/refractory FL patients in complete response, partial response or stable disease 2 months after a standard 4-week course of rituximab were randomized 1:1 to receive Id-KLH (Mitumprotimub-t) plus GM-CSF or control (Placebo+GM-CSF) vaccine. Patients were immunized six times monthly, and then six times every other month, and then every 3 months until disease progression. The primary efficacy endpoint was time to progression. After a median follow up of 40 months, this trial showed no improvement in time to progression with Mitumprotimub-t plus GM-CSF when compared with control arm (Freedman et al. 2009).

Several differences in the above trials likely account for their disparate results. First, only patients who achieved complete remission were randomized in the BiovaxID clinical trial, whereas the MyVax trial included patients in partial response and the Mitumprotimub-t trial included patients in partial and stable disease. Moreover, the choice of a doxorubicin-containing chemotherapy regimen likely resulted in a higher proportion of patients achieving complete remission in the BiovaxID trial. Results from these trials suggest that a minimal residual disease state may be required for the vaccine-induced immune responses to be effective. Second, BiovaxID was prepared using the heterohybridoma method whereas MyVax and Mitumprotimub-t are recombinant proteins, suggesting that the immunogenicity of the idiotype vaccines may be different between the different formulations. Finally, it is possible that B cell depletion, resulting from the use of rituximab in the Mitumprotimub-t trial, may have had a deleterious effect on subsequent vaccine efficacy. Taken together, we speculate that minimal residual disease status, as exemplified by complete clinical remission, appears to be necessary for the clinical antitumor effect of idiotype vaccines, as demonstrated by the success of BiovaxID in a pivotal phase III trial.

5 Idiotype-Specific T Cell Immunity Elicited by Idiotype Vaccination in Preclinical Models

There are about ten billion different immunoglobulins in humans (Fanning et al. 1996). The acquisition of such huge repertoire of antibodies in one individual is achieved through the mechanism of V(D)J recombination and somatic hypermutation (Butler 1997). The highly variable region of an antibody that mediates the binding of antibody with antigen is composed of multiple domains (Fig. 1) (Berman et al. 1986). Each domain of antibody is encoded by genes that were selected from a large chromosome region. For example, the heavy chain variable region contains at least 55 different variable domain (*V*) genes, plus 27 diversity (*D*) genes, and six joining (*J*) genes that are located together on chromosome 14 (Fanning et al. 1996). The Ig light chain contains 124 IGLV subgroups for lambda light chain on the chromosome 22 and 200 IGKV subgroups for kappa light chain on the chromosome 2 in humans. These *V*, *D*, *J* genes and IGLV and IGLK subgroups are all candidates for the synthesis of heavy and light chain of idiotype protein (Dudley et al. 2005). *V*, *D*, *J* genes and IGLV and IGKV subgroups are selected by cell-type specificity, intra- and inter-locus ordering, and allelic exclusion mechanism and joined together by recombinases, to ensure that every antibody generated is different. After V(D)J recombination, some antibodies undergo further somatic hypermutation in the highly variable region to generate new antibodies for new antigens. Somatic hypermutation only affects the individual B cells and is not transmitted to offspring B cells. Because of V(D)J recombination and somatic hypermutation, it is ensured that there are enough antibodies to recognize and eliminate different types of

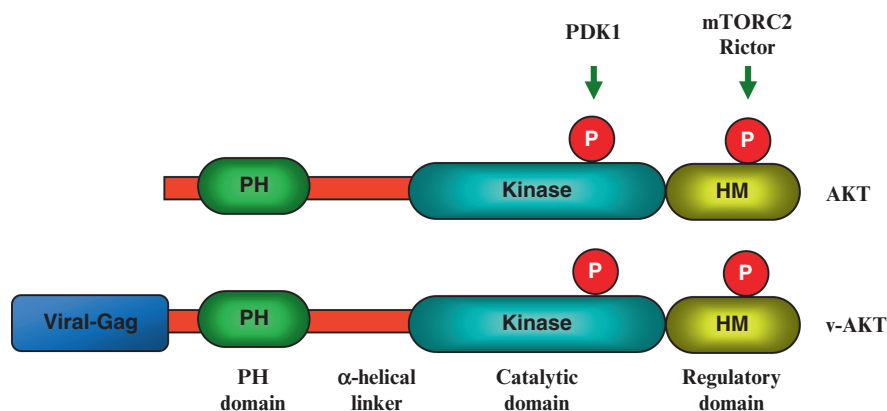


Fig. 1 The term idiotype refers to the unique amino acid sequences within the highly variable regions of the heavy and light chains of the surface immunoglobulin expressed on B cell malignancies. Idiotype includes both three complementarity-determining regions (CDR) and three framework region (FR) of immunoglobulin

antigens in human body. These processes also ensure that each malignant B cell expresses a unique idiotype protein.

Previous studies in mice have shown that idiotype proteins can be engulfed and processed through the exogenous pathway and presented in MHC I and II molecules (Weiss and Bogen 1991). Idiotype protein synthesized in the cytoplasmic compartment can also be processed and presented through the endogenous pathway (Yi et al. 1997). Idiotype-specific T cells can recognize the idiotype epitope derived from idiotype proteins on the cell surface of tumor B cells (Yi et al. 1993). These findings indicate that the idiotype protein can be processed and presented as a target for T cell recognition.

The search for cytokines and experimental adjuvants in preclinical models that could enhance idiotype-specific tumor immunity further suggested a role for T cells. The use of free granulocyte/macrophage colony-stimulating factor (GM-CSF) as an adjuvant drastically enhanced the cellular immune response of Id-KLH vaccination (Kwak et al. 1996). In this study, they investigated that low doses of GM-CSF 10,000 units i.p. or locally s.c. daily for 4 days significantly enhanced protective antitumor immunity induced by s.c. Id-keyhole limpet hemocyanin (KLH) immunization. This effect was critically dependent upon effector CD4 and CD8 T cells and was not associated with any increased anti-idiotypic antibody production. Specifically, 50% of mice immunized with Id-KLH plus GM-CSF on the same day of tumor challenge remained tumor-free at day 80, compared with 17% for Id-KLH alone, when immunization was combined with cyclophosphamide. Depletion of CD4 or CD8 T cells by using anti-CD8 or anti-CD4 antibody drastically decreased the protective role of idiotype vaccine (Kwak et al. 1996). It was likely that the mechanism of GM-CSF in the enhancement of vaccination was related to dendritic cell attraction and activation by GM-CSF (Wadhwa et al. 2003). This observation demonstrated that Id-KLH vaccines can stimulate a strong T cell immune response when combined with appropriate adjuvants.

Similarly, it was demonstrated that antitumor idiotype immune responses could be greatly enhanced by administration of myotoxins at the vaccine sites (Qin et al. 2009). In the study, ten BALB/c mice per group were injected intramuscularly with 6.8 μ g cardiotoxin (A) or 0.1 μ g crotoxin (B) followed by intramuscular vaccination at the same site 5 days later with 50 μ g plasmid DNA encoding MCP3 chemokine-fused A20 lymphoma-derived idiotype antigen (MCP3-sFv). TLR agonists, including TLR3 agonist Poly I:C, TLR4 agonist MPL, TLR7 agonist (M001), and TLR7/8 agonist (M003), respectively, were given on the next day of vaccination at a dose of 50 μ g. A total of three vaccinations were given with an interval of 14 days. Two weeks after final vaccination, all mice were challenged with a lethal dose of 2×10^5 A20 lymphoma cells by intraperitoneal injection and were followed for survival for 80 days. The authors found that administration of myotoxins at vaccination sites significantly enhanced idiotype DNA vaccine-induced tumor protection. About 90% mice from myotoxin treated group survived over the 80 days compared to only 10% mice, which survived in the control group. These data were also confirmed with two other chemotactic peptide-fused idiotype DNA vaccines with either defensin2 β - or MIP3 α -fused antigen. DNA vaccine

combinations with Poly I:C (TLR3 agonist), MPL (TLR4 agonist), M001 (TLR7 agonist), and M003 (TLR7/8 agonist), respectively, failed to enhance tumor protection. Both memory and therapeutic antitumor immunity were enhanced by the use of myotoxin, as more than 80% of mice protected by combination DNA vaccine plus cardiotoxin were resistant to tumor rechallenge, compared with less than 40% of mice protected by DNA vaccine alone ($P = 0.01$). Importantly, the authors found that the protective idiotype immunity was mediated by T cells, because depleting CD8⁺ T cells in vivo after vaccination plus cardiotoxin was clearly associated with reduced tumor protection, and depletion of both CD4⁺ and CD8⁺ T cell subsets abrogated protection completely. Of primary relevance, this study was the first to show that humoral immunity, however, was dispensable for anti-tumor immunity, as DNA vaccine plus cardiotoxin protected both genetically B cell-deficient J_h mice and wild-type mice equally from tumor challenge. More than 80% of tumor-free J_h mice surviving from the primary challenge were surprisingly highly resistant to tumor rechallenge, which suggests that anti-idiotype antibodies did not contribute principally to memory antitumor immunity. The memory antitumor immunity developed in J_h mice was comparable to that found in vaccinated wild-type counterparts.

6 Human T Cell Epitopes Identified in Lymphoma-Derived Idiotypic Proteins

Although initial human studies suggest that idiotype-specific T cells can be generated by vaccination, the precise antigenic determinants, or T cell epitopes of idiotype protein, are only partially characterized. The structure and sequence of each idiotype protein is different, and T cell epitopes usually represent a 8–15-amino acid long sequence. These factors make the characterization of idiotype epitope extremely time consuming and expensive. However, characterization of T cell epitopes derived from human idiotype proteins would be highly significant. First, we may be able to identify universal idiotype epitopes shared by patients, thus relieving the effort to prepare idiotype vaccine for each patient. Second, peptide-tetramers can be synthesized and used to monitor T cell immunity in idiotype vaccinated-patients. Third, idiotype peptides could be used to develop novel peptide-vaccine strategies. Finally, idiotype peptides may be used to selectively expand idiotype-specific T cells in vitro for adoptive T cell transfer, as peptides are much more effective antigen for T cell stimulation than the intact idiotype protein (Hansson et al. 2003).

Trojan et al. (2000) combined bioinformatics and in vitro T cell expansion system to identify human immunoglobulin-derived peptides capable of inducing cytotoxic T lymphocyte responses. They cloned 128 heavy chain and 35 light chain sequences from 192 patients and synthesized 794 peptides from 65 patients that were HLA-A2 positive. Among these peptides, 229 (28.8%) were derived from the

complementarity-determining regions (CDRs) of the immunoglobulin and the remaining 565 peptides (71.2%) were derived from framework region (FR) motifs. Because of the high homology of amino acid sequences in FR, 29.5% FR-derived peptides were shared by patients. The authors then stimulated purified CD8 T cells from HLA-A2 positive healthy donors with irradiated monocyte-derived peptide-pulsed dendritic cells and restimulated with irradiated, peptide-pulsed, CD40-activated B cells. They found nine FR-derived and one CDR-derived peptide that could stimulate CTLs from healthy donors. Five idiotypic peptide-stimulated CTLs could kill both the peptide-pulsed CD40-activated B cells and CLL cells that expressed this peptide. Three peptides could stimulate both allogenic and autologous CTLs (Trojan et al. 2000). This study represents the first large scale and detailed characterization of CD8 T cell epitopes in idiotype protein. Although the percentage of T cell epitopes found in the study was quite low, a framework region (FR3) derived CD8 T cell epitope was found to be shared by patients. This means that one idiotype-peptide specific CTLs may potentially target tumors from several patients. By employing a strategy using heteroclitic peptides, the authors found additional CD8 T cell epitopes from idiotype protein and in their subsequent studies, confirmed that idiotype protein is an immunogenic antigen (Trojan et al. 2000; Harig et al. 2001; Zirlik et al. 2006).

Another important study identifying human T cell epitopes in idiotype protein came from the NCI phase II clinical trial. Instead of using naïve T cells from healthy donors, Baskar et al. (2004) used peripheral blood mononuclear cells (PBMC) from idiotype protein-vaccinated patients. By T cell proliferation and cytokine secretion assays, using that postvaccination patients' PBMC, they demonstrated recognition of multiple CD4 and CD8 T cell epitopes that were exclusively located in the CDRs of idiotype protein. The idiotype-specific CD4 T cells in this study recognized the idiotype protein-pulsed autologous PBMC and HLA-matched EBV B cell lines in an MHC II-dependent manner. Interestingly, the idiotype CD4 T cells recognized the idiotype epitope on multiple HLA class II alleles, including MHC II DR, DQ, and DP, indicating the proximal binding ability of idiotype epitope on the MHC class II molecules. The CD8 T cell line isolated from a post vaccine patient recognized the epitope on the HLA-B08 allele only. Through peptide mapping experiments, they found the minimal determinant of an immunodominant epitope to be composed of critical amino acid residues that may be a product of somatic hypermutation (Baskar et al. 2004). This study confirmed the long-held belief that idiotype vaccination can induce both CD4 and CD8 T cell responses and that there are CD4 and CD8 T cell epitopes in idiotype protein (Bendandi et al. 1999). However, the mapping of most T cell epitopes to the CDR2 and CDR3s of idiotype protein was different from the predominant localization of epitopes to the FR regions by Trojan et al. (2000).

The issue of whether the CDR or FR regions of idiotype protein contribute more T cell epitopes was addressed by another group in Europe, which showed that the CDRs of patients contained more HLA-binding epitopes than the FRs. By using bioinformatics and molecular analysis section (BIMAS) and SYFPEITHI, two online programs, Hansson et al. (2003) found 456 HLA-binding peptides from the CDR

but only about 233 HLA-binding epitopes from the FR of the idiotype protein. They also found that most of the predicted peptides were confined to the CDR2-FR3-CDR3 “geographic” region of the Ig-VH region (70%), and that significantly fewer peptides were found within the flanking (FR1-CDR1-FR2 and FR4) regions ($P < 0.01$). Naturally occurring T cells in the myeloma patients’ blood recognizing the CDR-derived peptide was found by ELISPOT assay.

Other smaller studies from other groups have tried to characterize the T cell epitopes from the idiotype protein. Wen and Lim (1997) found the CDR3 of the heavy chain of idiotype proteins to contain a strong immunogenic epitope that can stimulate T cell proliferation, cytokine secretion, and cytotoxic activity. Dabadghao et al. (1998) study found that dendritic cells pulsed with idiotype heavy chain are much more immunogenic than dendritic cells pulsed with idiotype light chain, so they hypothesized that idiotype heavy chain may contain more epitopes than the light chain. In the study by Fagerberg et al. (1999), candidate peptides from the heavy chain of idiotype protein responded to the idiotype-specific T cells. In contrast, peptides from idiotype light chain could not be recognized by idiotype-specific T cells, so they concluded that idiotype light chains contain no T cell epitopes.

7 Ig Light-Chain as a Source of Idiotype Peptide Recognized by Human T Cells

The search for T cell epitopes in idiotype proteins will continue to be an area of interest for tumor immunologists, because the identification of immunogenic epitopes can serve as a gateway for developing subsequent immunotherapy, as described earlier. Studies have shown that there is preferential usage of specific variable, diversity, and joining genes at different stages of B cell development and in B cell malignancies (Li et al. 2004a). We propose that there are T cell epitopes in both FR and CDR of idiotype proteins, and the idiotype epitope can stimulate both CD4 and CD8 T cells. One limitation of the studies to date is that they have focused mostly on the heavy chain of idiotype proteins. It is generally believed that the heavy chain contains highly variable diversity-joining parts (VH-D-JH) in the CDR3, which is more immunogenic to T cells (Cohen et al. 2009). However, the idiotype light chains are not well studied to date. Since the highly variable region of idiotype protein contain both heavy chain and light chain and the idiotype light chain are also generated through V(D)J recombination and somatic hypermutation process, it is plausible that the idiotype light chain also contains T cell epitopes.

Recently, we have tried to answer this question with human U266 myeloma cell line and PBMC from healthy donors. The idiotype light chain of U266 was cloned and sequenced with primers published previously (Tiller et al. 2008). Fifteen peptides corresponding to idiotype light chain of U266 idiotype protein were selected based on the binding capacity to HLA-A2 molecules. The peptides were then dissolved in DMSO and used to stimulate PBMC from an HLA-A2-positive

healthy donor by using a published protocol (Hida et al. 2002). Briefly, PBMCs (1×10^5 /well) obtained from HLA A2 positive healthy donors were incubated with 10 μ g/ml (10 μ M) peptide in 96-well plate in the presence of 100 U/ml interleukin-2 at day 0 with media that consist of 50% RPMI1640 and 50% AIM-V from Invitrogen. At culture days 4, 7, 10, and 13, half of the cultured media were removed and the cells were restimulated with 10 μ g/ml of the peptide. At day 15, the cultured cells were resuspended in fresh medium and mixed with T2 cells loaded with corresponding peptide. T2 cells loaded with HIV peptide were used as a control. After 18 h of coculture, the concentration of IFN- γ in the culture supernatants was collected and measured by ELISA. As shown in Fig. 2, the U266 idiootype light chain-derived peptides P28 (GVTISCSGST) stimulated T cells that secreted a large amount of IFN- γ when cocultured with T2 loaded with P28 peptide, indicating the presence of peptide-specific T cells. The peptide-stimulated T cells were further expanded and analyzed by cytotoxic assay. T2 cells loaded with 40 μ M idiootype light chain peptide P28 in the presence of 3 μ g/ml β 2-microglobulin were labeled with chromium-51 and cocultured with P28 peptide stimulated T cells at different concentrations. As shown in Fig. 3, the P28 peptide stimulated CTLs that can efficiently kill T2 cells loaded with P28 peptide but not T2 cells loaded with HIV peptide, indicating that these T cells are peptide-specific CTLs (Fig. 3). To test

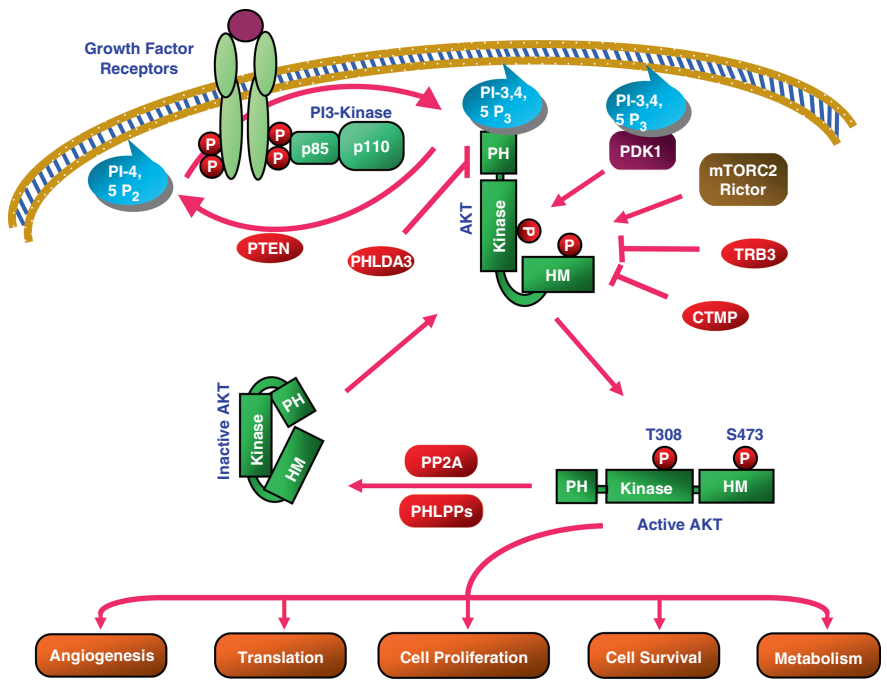


Fig. 2 IFN- γ ELISA. 1×10^5 idiootype light chain peptide-specific T cells secreted a large amount of IFN- γ when cocultured with 1×10^5 T2 cells loaded with 10 μ M corresponding peptide (P28) compared with T2 loaded with 10 μ M HIV peptide

Fig. 3 T2 cell cytotoxic assay. Idiotype light chain peptide (P28)-specific T cells efficiently kill T2 cells loaded with 40 μ M corresponding peptide (P28) compared with T2 loaded with 40 μ M HIV peptide

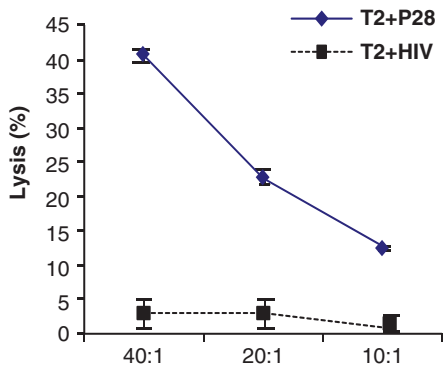
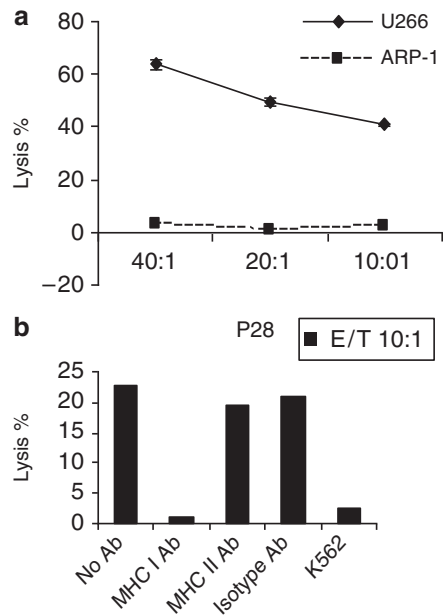


Fig. 4 U266 myeloma cytotoxic assay. (a) Idiotype light chain peptide (P28)-specific T cells efficiently kill U266 myeloma cells but not another myeloma (ARP-1 cells), which is HLA-A2 negative. (b) The killing of U266 by idiotype light chain peptide (P28)-specific T cells was blocked by MHC class I antibody but not MHC class II antibody, indicating the epitope was presented with MHC class I allele. The idiotype light chain peptide (P28)-specific T cells do not kill K562 cells which are a target of NK cells



whether the peptide was processed and presented by U266 myeloma cells, we cocultured the T cells with U266 myeloma cell at different concentrations. As shown in Fig. 4, idiotype light chain peptide stimulated T cells that can efficiently kill U266 tumor cells but not the control human myeloma ARP-1 cells, indicating that the idiotype light chain peptide was processed and presented on the cell surface of U266 cells. To determine whether the epitope was conjugated with the MHC allele of U266 cells, we performed an MHC antibody blocking assay. U266 myeloma cells were labeled with chromium-51, washed, and incubated with MHC antibodies for 1 h before coculturing with P28 CTLs. As shown in Fig. 4, the killing of U266 by P28 peptide-specific CTLs was blocked by MHC class I

antibody but not by MHC class II antibody, indicating that the epitope was presented with the MHC class I allele of U266 cells (Fig. 4). Our preliminary studies have expanded the repertoire of T cell epitopes in idiotype protein. In addition, it is known that about 20% myeloma tumors have lost heavy chain expression; therefore, CTLs targeting the idiotype light chain epitope may be of great value to this group of patients (Cohen et al. 2009). The cytotoxic effect of idiotype light chain of CTLs is being further tested in an *in vivo* system in our laboratory now.

Somatic hypermutation is a mechanism of B cells that is used to further increase antibody diversity and to adapt to new foreign agents. Somatic hypermutation occurs in the immunoglobulin variable region at a rate of 10^{-3} mutations per base pair per cell division, which is 10^6 -fold higher than the spontaneous mutation rate in somatic cells (Li et al. 2004b). The mechanism of somatic hypermutation involves the deamination of cytosine to uracil in DNA by an enzyme called activation-induced (cytidine) deaminase, or AID (Larson and Maizels 2004). The mismatched uracil:guanine pair are then removed by uracil-DNA glycosylase and filled by error-prone DNA polymerases to create mutations in the highly variable region of idiotype protein (Teng and Papavasiliou 2007). On the one hand, mis-regulated somatic hypermutation has been found to play a role in the development of B cell lymphomas and myeloma (Odegard and Schatz 2006). On the other hand, somatic hypermutation is also found to contribute to autoimmunity in humans (Atassi and Casali 2008). In the tumor immunity, germline cell lymphomas have been found to be more aggressive than lymphomas with somatic hypermutation (Hamblin et al. 1999; Maurer et al. 2005). Naturally occurring CTLs isolated from patients recognized peptides encompassing the somatic hypermutation site (Rezvany et al. 2000). In studies by Baskar et al. (2004) and Hansson et al. (2003), somatic hypermutation may have generated T cell epitopes. Replacement or deletion of the somatic hypermutation resulted in the loss of recognition by T cells. These observations indicate that somatic hypermutation may play an important role in T cell epitope generation. In our recent studies, we have identified idiotype T cell epitopes with somatic hypermutations, but epitopes without somatic hypermutation were also identified (data not shown). This indicates that both mutated and unmutated sites of idiotype protein may generate epitopes recognized by CTLs. Since somatic hypermutation affects only individual B cells and the mutations are not transmitted to offspring, the targeting of tumor cells via somatic hypermutation may be a patient-specific strategy.

8 Future Directions for Idiotype-Specific T Cell Immunotherapy

For 30 years, idiotype vaccination has been shown to generate a protective immune response against lymphoma and myeloma tumors, mainly in preclinical models. Future directions will focus on how to improve the effect of the T cell response

generated by idiotypic vaccination in human patients. As shown by the example of the idiotypic-KLH-GM-CSF vaccine, an effective vaccine will depend on three factors: tumor antigen, carrier molecules, and vaccine adjuvant. Because KLH has a large molecular weight and is more immunogenic than other molecules, it has become the most widely employed carrier protein for idiotypic vaccination. Recently, the alternative conjugation using the maleimide-based method has been reported to significantly enhance the immunogenicity of both human and murine Id-KLH vaccines in preclinical studies (Kafi et al. 2009). The mechanism of GM-CSF in the enhancement of T cell response in idiotypic vaccination involved dendritic cell differentiation, migration, and maturation by GM-CSF through the STAT5 signaling pathway (Esashi et al. 2008). Dendritic cells are the most potent antigen presenting cell (APC) to induce antigen-specific T cells *in vivo*. Recent studies demonstrated that Toll-like receptor signaling pathways also play important roles in the differentiation, maturation, and activation of dendritic cells (Biragyn et al. 2002; Schjetne et al. 2003). Activation of Toll-like receptor signaling by CpG strongly enhances dendritic cell-mediated vaccine efficiency (Speiser et al. 2005). How to incorporate the growing knowledge of Toll-like receptor signaling pathway and dendritic cell activation into our idiotypic T cell generation will be important for idiotypic vaccination.

The observation that over 50% cancer patients can respond to the adoptive T cell transfer has made the adoptive immunotherapy one of the most attractive strategies (Dudley et al. 2002). A previous study has demonstrated that it is feasible to transfer idiotypic-specific T cells from healthy donors to recipients (Kwak et al. 1995). The transferred idiotypic-specific T cells protected mice against established tumor in mouse model (Hornung et al. 1995). T cells can also be generated in large number *in vitro* for adoptive T cell transfer. It has been demonstrated that the success of T cell transfer will depend heavily on the quality of transferred T cells. T cells that belong to central memory (T_{CM}) type but not terminal differentiated type (T_{EM}) can proliferate well and exert a protective role after adoptive transfer (Wherry et al. 2003; Gattinoni et al. 2005). Generating T cells that have a high percentage of T_{CM} cells is essential for the success of adoptive T cell transfer. IL-15 and IL-7 have been reported to increase the percentage of T_{CM} cells in the T cell population (Ku et al. 2000; Schluns et al. 2000; Tan et al. 2002). Recent studies also have demonstrated CD28 and CD27 expression and 4-1BB ligand interaction to increase the pool of T_{CM} cells (Acuto and Michel 2003; Oelke et al. 2003; Topp et al. 2003). Telomeres of T_{CM} cells are characteristically longer than those of T_{EM} cells, which make T_{CM} capable of going through multiple cell divisions (Weng et al. 1997; Rufer et al. 2003). IL-15 is capable of activating telomere synthesis in memory CD8 T cells via Jak3 and PI3K signaling pathways (Li et al. 2005). Further studies of the T cell population will help us to achieve the best culture conditions for preparing idiotypic specific T_{CM} cells for adoptive T cell transfer. Moreover, CD4 T cells have proven to be able to improve and expand CD8 T cell function through cytokine- and CD40L-dependent pathways (Bevan 2004). Adoptive transfer of both CD4 and CD8 T cells produced a greater effect than single CD8 T cell transfer

(Ossendorp et al. 1998). Therefore in the future, idiotype-specific adoptive T cell transfer may involve both CD4 and CD8 T cells.

A healthy microenvironment has been demonstrated to be extremely important for the T cell response *in vivo*. For example, an increase in CD4+CD25+ Treg cell has been observed in lymphoma and myeloma patients (Beyer et al. 2006; Yang et al. 2006). In normal healthy donors, this group of T cells can suppress the autoimmunity mediated by T cells. However, in a tumor environment, this group of T cells can inhibit the immune response of tumor-specific T cells. In both human and mouse studies, it has been shown that the depletion of Treg can drastically increase the antitumor effect of immune system (Sutmuller et al. 2001; Dannull et al. 2005). Moreover CTLs have been reported to express inhibitor molecules, such as PD-1, CTLA4, and B7-1. These molecules can interfere with the cytotoxic function of CTLs, and the inhibition of these molecules has been shown to improve the antitumor immunity by tumor antigen-specific CTLs (Sutmuller et al. 2001; Iwai et al. 2002; Curiel et al. 2003). Taken together, these studies demonstrate that the combination therapy of active immunization and adoptive T cell transfer, together with immune suppressive pathway inhibition, may be the ideal immunotherapy strategy for hematologic malignancies in the future.

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Erratum: Identification of Human Idiotype-Specific T Cells in Lymphoma and Myeloma

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The original version of this article unfortunately contained two mistakes. Figures 1 and 2 were incorrect. The correct versions are given below:

Fig. 1 The term idiotype refers to the unique amino acid sequences within the highly variable regions of the surface immunoglobulin expressed on B cell malignancies. Idiotype includes both three complementarity-determining regions (CDR) and three framework region (FR) of immunoglobulin

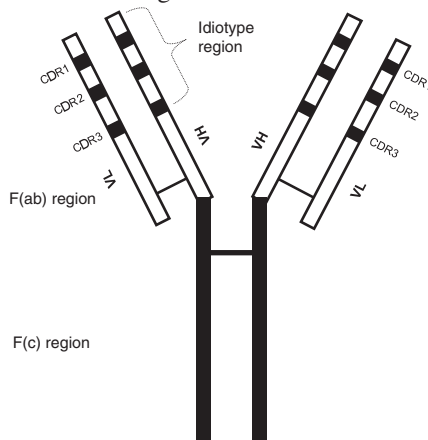
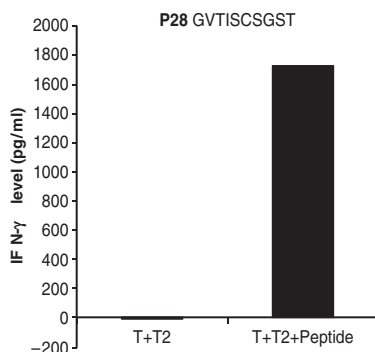


Fig. 2 IFN- γ ELISA. 1×10^5 idiotype light chain peptide-specific T cells secreted a large amount of IFN- γ when cocultured with 1×10^5 T2 cells loaded with $10 \mu\text{M}$ corresponding peptide (P28) compared with T2 cells loaded with $10 \mu\text{M}$ HIV peptide



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