Targeting lymphoma with precision using semisynthetic anti-idiotype peptibodies

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B-cell lymphomas express a functionally active and truly tumor-specific cell-surface product, the variable region of the B-cell receptor (BCR), otherwise known as idiotype. The tumor idiotype differs, however, from patient to patient, making it a technical challenge to exploit for therapy. We have developed a method of targeting idiotype by using a semisynthetic personalized therapeutic that is more practical to produce on a patient-by-patient basis than monoclonal antibodies. In this method, a small peptide with affinity for a tumor idiotype is identified by screening a library, chemically synthesized, and then affixed to the amino terminus of a premade IgG Fc protein. We demonstrate that the resultant semisynthetic anti-idiotype peptibodies kill tumor cells in vitro with specificity, trigger tumor cell phagocytosis by macrophages, and efficiently clear human lymphoma in a murine xenograft model. This method could be used to target tumor with true precision on a personalized basis.

lymphoma | peptibody | idiotype | precision medicine

Combination chemotherapy administered with an anti-CD20 monoclonal antibody is the mainstay of treatment for non-Hodgkin lymphoma (NHL). Although impressive, responses are often seen in the aggressive lymphomas, the majority of patients with indolent lymphomas relapse and become insensitive to treatment, ultimately succumbing to their disease (1). Treatment with chemotherapy can result in serious complications including end-organ damage and secondary malignancies (2). Additionally, chemotherapies and anti-CD20 monoclonal antibodies are immunosuppressive, leaving patients susceptible to infection (3) and potentially blunting adaptive antitumor immune responses (4). A more targeted therapy using a novel mechanism of tumor clearance would be a welcome addition to the arsenal available for NHL management.

Idiotype is unique to each clone of malignant cells in B-cell lymphomas and is distinct from the homologous structure present on nonmalignant B cells (Fig. 1*A*). Anergy and clonal deletion can ensue when naive B cells receive a B-cell receptor (BCR) stimulus in the absence of T-cell help (5), Toll-like receptor ligation (6), or appropriate cytokine signals from accessory cells (6). Therefore, idiotype is both a truly tumor-specific and functionally active cell surface marker capable of triggering apoptosis. Antibodies against idiotype can induce complete regression of lymphoma in patients (7–10). Unlike most anti-tumor antibodies, anti-idiotype antibodies appear to clear tumor primarily through a direct effect on tumor cells mediated by the induction of BCR signaling (11) rather than through antibody-dependent cellular cytotoxicity or phagocytosis (12). However, despite its inherent specificity, efficacy, and novel mechanism of action, anti-idiotype therapy requires the generation of a custom monoclonal antibody for each patient, thus rendering it impractical.

Here, we describe an alternate strategy for targeting tumor idiotype that does not require custom cloning or the biologic production of a unique macromolecule for each patient. Short idiotype-binding peptides can be identified for each tumor-derived idiotype by high-throughput screens of oligopeptide libraries (13–16) and, when synthesized as multivalent tandem repeats, these peptides can cluster BCR and trigger signaling that results in apoptosis of lymphoma cells in vitro (17). Because they can be produced rapidly and inexpensively by solid-phase synthesis, idiotype-binding peptides might represent a practical anti-idiotype therapy; however, they are cleared quickly from serum, as is typical for small peptides, and are therefore not sufficient to induce tumor clearance in vivo. To overcome this limitation, we designed a system to effect a chemical fusion between idiotype-binding peptides and a recombinant Ig Fc domain, yielding a semisynthetic peptibody (Fig. 1*B*). This modular design allows for the synthetic production of the patient-specific oligopeptide and bulk biologic production of the patient-invariant biologic Fc domain. The presence of an IgG Fc domain not only improves the pharmacokinetics of idiotype-binding peptides, but also adds an additional mechanism of tumor clearance through tumor cell opsonization and activation of innate immune effector cells (Fig. 1*A*).

Results

We used native chemical ligation (NCL), a method of site-selective polypeptide ligation, to covalently affix idiotype-binding peptides to the Fc domain of Ig (18). To facilitate coupling, we first engineered a recombinant murine IgG2a Fc domain bearing an amino-terminal cysteine (Fig. S1 *A* and *B*). A previously identified idiotype-binding peptide for the SUPB8 human lymphoma (17) was produced with a carboxyterminal thioester incorporated during synthesis. These species were then reacted in the presence of a sodium mercaptoethane sulfonate (MESNA) catalyst, and the resultant semisynthetic peptibody was purified by affinity chromatography (Fig. S2). We produced two different functional peptibody constructs, one linked to two copies of the idiotype-binding peptide in tandem and one with a single copy of the peptide (Fig. S1 *C* and *D*). These two constructs, when assembled into an Ig-like

Significance

Idiotype is a true tumor-specific surface marker on lymphoma cells, and cross-linking idiotype can directly trigger lymphoma cell death. It has not been practical, however, to target idiotype with antibodies because its sequence is unique to each patient. By chemically linking short synthetic patient-specific idiotype-binding peptides to a patient-invariant IgG Fc domain, we created a modular, antibody-like molecule that is more practical to customize. These semisynthetic peptibodies kill lymphoma cells directly, trigger tumor cell phagocytosis by macrophages, and clear human lymphoma in a mouse model of disseminated disease. This technology could add a new mechanism of action to the armament of lymphoma therapies and would not possess the toxicities and immunosuppression inherent to the current standard of care.

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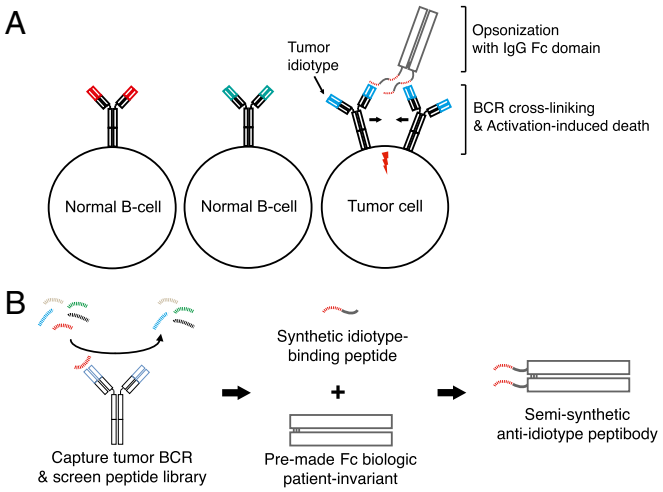

Fig. 1. Concept overview. (*A*) Tumor idiotype is the variable region of the BCR on the surface of lymphoma cells. It is unique to the tumor clone and distinct from the idiotype on other B-cell clones. Peptibodies cross-link idiotype, triggering BCR signaling, resulting in activation-induced cell death, and opsonize tumor cells with an IgG Fc domain, promoting tumor clearance mediated by innate immune effector cells. (*B*) Schematic of semisynthetic anti-idiotype peptibody production. A random peptide library is screened to identify peptide sequences with idiotype-specific binding, which are synthesized and affixed to the amino terminus of a premade recombinant IgG Fc domain.

dimer, display tetravalent and bivalent binding, respectively, to the idiotype targets of tumor B cells. We also produced a nonfunctional peptibody with scrambled idiotype-binding sequences. In various assays, the tetravalent peptibody was compared with the bivalent form, to a monoclonal anti-idiotype antibody against the same idiotype target, to a generic anti-IgM monoclonal antibody, or to the anti-CD20 antibody rituximab.

To characterize the functional properties of the peptibody, we first evaluated its binding specificity and avidity. The peptibody bound both on-target purified idiotype (Fig. 2*A*) and lymphoma cells (Fig. 2*B*) with nanomolar avidity, but did not bind isotype matched off-target lymphoma cells (Fig. 2*C*).

We next asked whether the peptibody was able to cluster the lymphoma cell BCR and trigger signaling, which is necessary for the induction of apoptosis by idiotype-binding peptides. The peptibody and the lymphoma cell BCR began to colocalize and cluster on the lymphoma cell surface within 1 h of treatment, and a unipolar cap of peptibody and BCR had formed by 12 h (Fig. 3*A*). Intracellular Syk and ERK phosporylation were stimulated in response to peptibody treatment, indicating activation of BCR signal transduction (Fig. 3*B*). This signaling was more robust in cells treated with peptibody tetramer than those treated with peptibody dimer or anti-idiotype monocolonal antibody.

We then investigated the ability of the peptibody to induce apoptosis and directly kill tumor cells. Peptibody treatment for 24 h resulted in caspase-3 cleavage (Fig. 3*C*) and apoptosis (Fig. 3*D*). A saturating dose of peptibody tetramer induced apoptosis in a larger percentage of cells than that of a peptibody dimer or an anti-idiotype monoclonal antibody. After 3 d of treatment, complete killing was observed in cells treated with peptibody tetramer with a half-maximal effect in the nanomolar range, similar to that observed when cross-linking BCR with an anti-IgM monoclonal antibody (Fig. 3*E*).

Taken together, these in vitro studies demonstrate that anti-idiotype peptibodies bind with high avidity to target cells, activate BCR signaling, and trigger apoptosis and lymphoma cell death. Comparison of constructs indicates that the peptibody tetramer bound tumor cells with a higher avidity and induced apoptosis more potently than constructs with a lower binding valency, such as the peptibody dimer and anti-idiotype monoclonal antibody. We therefore used the peptibody tetramer in subsequent studies.

Having demonstrated a robust direct antitumor effect, we next evaluated the ability of the peptibody to activate macrophages and promote tumor cell phagocytosis. Mouse macrophages labeled with red fluorescent protein (RFP) were cocultured with tumor cells labeled with green fluorescent protein (GFP) and treated with saturating doses of peptibody, anti-idiotype monoclonal antibody, or rituximab. Phagocytosis was measured by the fraction of GFP/RFP double-positive macrophages (Fig. 4*A*). The peptibody triggered robust phagocytosis of on-target tumor cells but not off-target isotype-matched tumor cells (Fig. 4*B*). Peptibody-induced phagocytosis was more robust than that induced by anti-idiotype monoclonal antibody or by rituximab. Half-maximal induction of phagocytosis was observed in the low nanomolar range, consistent with the previously observed idiotype and tumor cell binding avidity of the peptibody (Fig. 4*C*). These results demonstrate that anti-idiotype peptibodies can potently mediate targeted induction of phagocytosis by macrophages and indicate that the ability of the recombinant IgG2a Fc domain to ligate and activate Fc gamma receptors on innate immune cells is preserved after the molecule has been subject to the chemical conditions of NCL.

We next evaluated the serum half-life of the peptibody in mice. CB-17 severe combined immunodeficient (SCID) mice were given i.p. injections of peptibody, and timed serum samples were assessed for IgG2a concentration and idiotype binding. Whereas idiotype binding peptides were rapidly degraded in mouse serum (Fig. S3*A*) and not detectable in the serum of treated mice, the serum half-life of the peptibody was ~24 h (Fig. S3 *B* and *C*). The Fc domain therefore extended the half-life of idiotype binding peptides to a duration suitable for

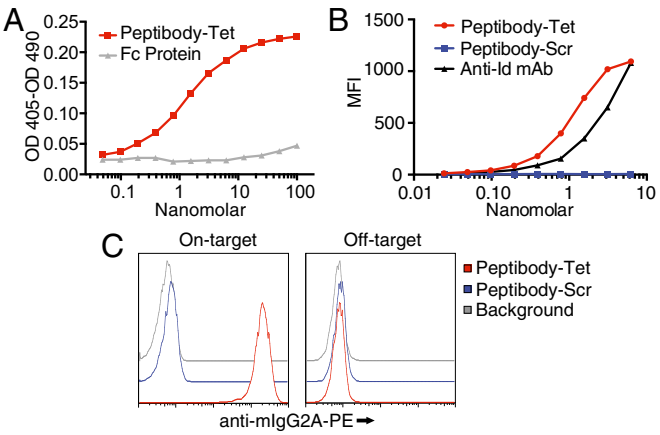

Fig. 2. Targeted idiotype and tumor binding. (*A*) Purified Ig from the on-target SUPB8 human lymphoma cells was immobilized on ELISA plates and exposed to varying concentrations of peptibody (Peptibody-Tet) or recombinant IgG2a Fc protein with no peptide attached. Binding was detected with an anti-mIgG2a-HRP antibody. The results are representative of at least four experiments. (*B*) On-target SUPB8 cells were exposed to varying concentrations of peptibody (Peptibody-Tet), peptibody with a scrambled idiotype peptide ligand sequence (Peptibody-Scr), or an anti-idiotype monoclonal antibody (Anti-Id mAb). Binding was detected with fluorophore-labeled anti-mIgG2a. Mean fluorescence intensity is plotted. The results are representative of at least four experiments. (*C*) On-target SUPB8 cells and off-target RAMOS cells with isotype-matched surface Ig were exposed to a saturating concentration of peptibody (Peptibody-Tet) or peptibody with a scrambled idiotype peptide ligand sequence (Peptibody-Scr). Binding was detected with fluorophore-labeled anti-mIgG2a. Results are representative of at least four experiments.

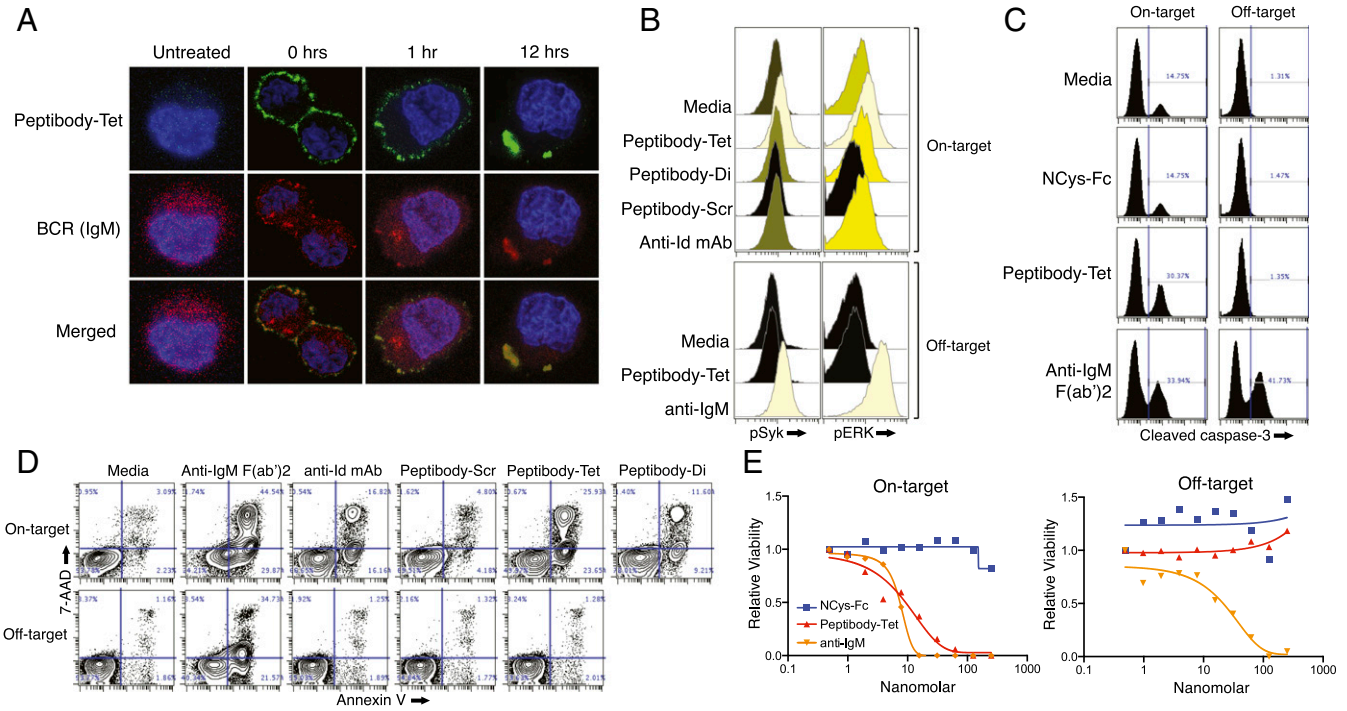

Fig. 3. Anti-idiotype peptibodies cluster BCR, directly trigger BCR signaling and caspase-3 cleavage, and directly trigger tumor cell apoptosis and death. (*A*) On-target SUPB8 cells were treated with a saturating 100-nM concentration of peptibody tetramer (Peptibody-Tet) for 0 h (20 min on ice), 1 h, or 12 h at 37 °C. Cells were fixed with paraformaldehyde, permeabilized with cold methanol, and stained with anti-mIgG2a-FITC to detect bound peptibody (green) and anti-hIgM-APC to detect BCR (red). Cells were imaged with confocal microscopy, and representative images are shown. (*B*) Cells were incubated with a saturating 100-nM concentration of peptibody tetramer (Peptibody-Tet), peptibody dimer (Peptibody-Di), scrambled peptibody (Peptibody-Scr), anti-idiotype mAb (Anti-Id mAb), or anti-IgM F(ab')2 for 5 min at 37 °C. Cells were fixed with paraformaldehyde, permeabilized with cold methanol, and stained with fluorophore-labeled anti-phospho Syk or anti-phospho ERK-1/-2. (*C*) Cells were incubated with mIgG2a Fc protein, peptibody, or anti-IgM Fab2′ for 24 h at 37 °C. Cells were fixed with paraformaldehyde, permeabilized with cold methanol, and stained with fluorophore-labeled anti-cleaved caspase-3. (*D*) Cells were incubated with 100 nM of peptibody tetramer, peptibody dimer, scrambled peptibody, anti-idiotype monoclonal antibody, or an anti-hIgM F(ab′)2 fragment for 24 h. Cell viability was assessed by 7-AAD exclusion, and surface phosphatidylserine expression was assessed by staining with FITC-labeled Annexin V. (*E*) Cells were incubated with varying concentrations of peptibody, mIgG2a Fc protein, anti-hIgM mAb, or anti-hIgG mAb for 3 d. Viability was assessed with the resazurin-based PrestoBlue dye. Fluorescence values were normalized to readings from untreated cells.

use as a therapeutic. The half-life as measured by serum IgG2a concentration was similar to that measured by idiotype binding, indicating that there was no differential proteolysis of the idiotype-binding sequence on the peptibody.

With the knowledge that the peptibody was stable in mouse serum, we assessed its in vivo antitumor activity. We challenged SCID mice i.v. with on-target tumor cells engineered to stably express firefly luciferase (SUPB8-Luc$^+$ cells). On day 2 following challenge, mice were treated with 65 μg of peptibody administered i.p., once daily, for 4 d. Complete tumor clearance occurred in all treated mice, whereas the tumor grew rapidly in the untreated mice (Fig. 5).

We then evaluated the ability of the peptibody to treat a more established tumor compared with that of an anti-idiotype monoclonal antibody, rituximab, or the control peptibody with scrambled idiotype-binding sequences. SCID mice were challenged i.v. with SUPB8-Luc$^+$ cells and, beginning on day 6 following challenge, mice were treated once daily with i.p. injections of 50 μg of the appropriate therapeutic for 8 d. Peptibody treatment lead to complete tumor clearance and recurrence-free survival in four of five mice, anti-idiotype monoclonal antibody treatment resulted in tumor clearance in three of five mice, and rituximab treatment resulted in tumor clearance in four of five mice (Fig. 5 *D* and *E*). Tumor grew rapidly in all mice that were treated with the nonfunctional scrambled peptibody. These experiments demonstrate that anti-idiotype peptibodies potently clear human lymphoma in vivo in a mouse xenograft model system.

Discussion

It has been more than 30 years since idiotype was recognized as a tumor-specific surface target, but despite encouraging results from early clinical trials with anti-idiotype antibodies, the impracticalities inherent to drugging a target with a sequence that varies on a patient-by-patient basis have prohibited further progress. We have adapted the peptibody format (19) for use as a platform for personalized anti-idiotype therapy by modifying the peptide-Fc fusion chemically rather than genetically. The advantages of using peptibodies as opposed to monoclonal antibodies for anti-idiotype therapy extend beyond the ease of personalization: peptibodies have a molecular weight one-third that of antibodies, which may allow for greater tissue permeability; the peptibody format allows a single molecule to bear more than two antigen-binding domains, which results in higher avidity for the target, and, as we demonstrated, this higher antigen-binding domain valency allows for more robust cross-linking and activation of functional target receptors such as idiotype.

Results from small clinical trials of anti-idiotype antibody therapy suggest that idiotype cross-linking and stimulation of the BCR was the primary mechanism of tumor clearance (11). Indeed, we find that cross-linking lymphoma idiotype with peptibodies results in activation-induced cell death. Both this strong, sustained BCR signaling and, conversely, the absence of BCR signaling (20), can trigger B-cell death. Although the inhibition of BCR signaling has been successfully used to treat B-cell chronic lymphocytic leukemia (21) and mantle cell lymphoma

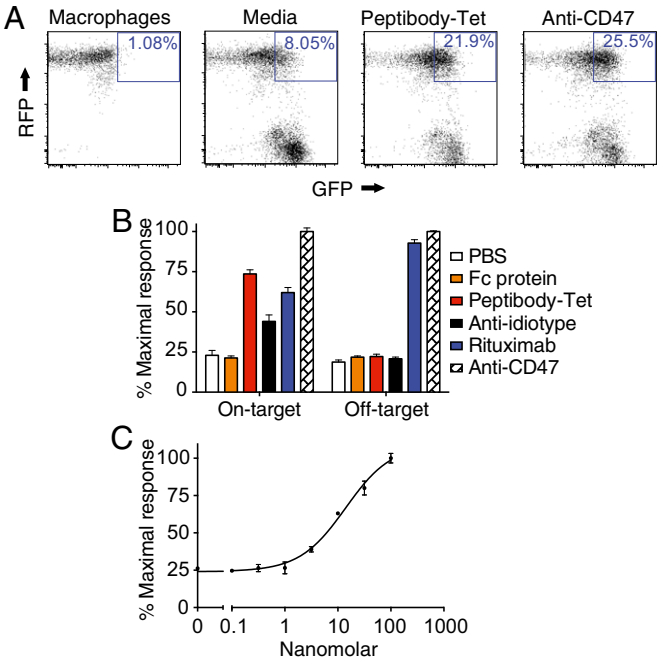

Fig. 4. Anti-idiotype peptibodies promote innate immune effector-mediated clearance in vitro. (*A*) RFP$^+$ mouse macrophages were incubated with GFP$^+$ SUPB8 cells or GFP$^+$ Raji cells in the presence of a saturating 100-nM concentration of peptide ligand, peptibody, mIgG2a Fc protein, anti-idiotype mAb, or rituximab. Representative FACS plots demonstrating RFP/GFP double-positive macrophages that have phagocytosed-labeled tumor cells. (*B*) Phagocytosis normalized to average % GFP$^+$ macrophages upon treatment with anti-CD47 for each cell line (% maximal response). (*C*) Phagocytosis of SUPB8 cells in response to varying concentrations of peptibody. Normalized to mean percentage of GFP$^+$ macrophages at saturating dose.

(22), activation-induced cell death has yet to be exploited for therapy. Moreover, anti-tumor antibodies, such as rituximab, do not clear tumor primarily through a direct effect on the tumor cell, but rather by engaging innate immune effector cells that mediate antibody-dependent cellular cytotoxicity and phagocytosis (23). It is not known at this time why some lymphomas cease to respond to rituximab treatment (24), and further investigation may shed light on whether these resistant tumors are sensitive to peptibody-mediated activation-induced cell death.

In addition to leveraging a novel mechanism of tumor clearance, anti-idiotype therapy would enable truly tumor-specific cytoreduction. Rituximab, by contrast, opsonizes all cells that express the tumor-associated antigen CD20 and mediates ablation of the entire B-cell compartment. The resultant immunosuppression puts patients treated with rituximab at an increased risk of infection and viral reactivation (3). Perhaps just as importantly, clearance of the B-cell compartment removes an antigen-presenting cell (APC) population that is important in the initiation of T-cell–mediated cellular immunity (25). Preservation of nontumor B cells could thus promote an adaptive immune response against the tumor.

In designing anti-idiotype peptibodies, our goal was an agent that would not only trigger activation-induced death of tumor cells by stimulating BCR signaling but also opsonize these cells and promote innate immune effector-mediated clearance. We therefore selected an Fc domain isotype capable of ligating activating Fc gamma receptors. Interestingly, recent work has shown that some antibody agonists require Fc domains capable of interacting with inhibitory Fc receptors when the target receptor is activated by clustering (26). Presumably, inhibitory Fc receptors on the surface of effector cells abutting target cells serve as a scaffold to facilitate more robust target receptor clustering. It is not clear that peptibodies would be subject to this requirement, however. For instance, the peptibody agonist of the thrombopoietin receptor, romiplostim, is active despite the use of a human IgG1 Fc domain (19). The higher valency of receptor ligands displayed on peptibodies and the improved 3D flexibility of these ligands may allow for robust target receptor cross-linking in the absence of stabilization by a scaffold.

Peptibody-mediated phagocytosis of tumor cells and presentation of tumor antigens by APCs such as macrophages or dendritic cells could also promote an adaptive immune response that might increase the probability of obtaining a durable remission (27–29). Because phagocytosis would be restricted to tumor cells that express tumor-specific neoantigens, peptibodies might promote a more robust adaptive immune response than antibodies against tumor-associated antigens such as CD20 or other agents that mediate less than tumor-specific cell death or phagocytosis. Anecdotal evidence from anti-idiotype antibody trials suggests that adaptive immunity may have been induced in some individuals, because long-duration disease-free remission was achieved in patients with follicular lymphoma, including in a patient whose tumor idiotype exhibited enough heterogeneity to preclude anti-idiotype antibody binding to a small fraction of tumor cells (30). There is renewed interest in agents that induce tumor-specific adaptive immunity given the efficacy of immune checkpoint blockade in eliciting existing adaptive immune responses (31). Combination immunotherapy with a truly tumor-specific peptibody and a checkpoint blockade inhibitor might result in more durable remissions than treatment with either agent alone.

In conclusion, we propose a method of targeting lymphoma idiotype on a patient-specific basis by using semisynthetic anti-idiotype peptibodies, a therapeutic that is more practical to personalize than monoclonal antibodies. These peptibodies kill tumor cells directly in vitro with specificity, stimulate tumor cell phagocytosis by macrophages, and efficiently clear human lymphoma in a murine xenograft model. This technology could facilitate the personalized precision therapy of B-cell lymphoma, and perhaps other diseases involving pathologic clonal B-cells populations, by targeting a functionally active and truly tumor-specific cell surface product that has long been recognized but has yet to be exploited for therapy.

Materials and Methods

Engineering and Production of a mIgG2a Fc Protein with an Amino-Terminal Cysteine. Because translated proteins begin with an amino-terminal methionine, it was necessary to generate a proprotein that is proteolytically cleaved by endoproteinases within the cell to reveal an amino-terminal cysteine. We used site-directed mutagenesis to remove nucleotide positions 573–653 from the pFUSE-mIgG2a-Fc2 vector (InvivoGen) and insert a human Ig kappa signal sequence and cysteine codon immediately 5′ to the mouse IgG2a Fc coding sequence (Fig. S1 *A* and *B*). Site-directed mutagenesis was performed by using the QuikChange II XL kit (Agilent Technologies). The engineered vector was electroporated into 293-F cells (Invitrogen) using an Amaxa Nucleofector. These cells were maintained under antibiotic selection to obtain stable transformants and subcloned to select for high transgene expression. For protein production, cells were grown in FreeStyle serum-free media (Invitrogen) and the NCys-mIgG2a Fc protein was purified from conditioned media by Protein A affinity chromatography.

Identification of the Idiotype-Binding Peptide Sequence. Detailed methods for the identification of idiotype-binding peptides by peptide phage-display screening, including identification of the SUPB8 idiotype-binding peptide used in this study, are referenced (16, 17). In brief, tumor idiotype was affinity purified from media conditioned by hybridomas generated from the fusion of SUPB8 tumor cells with the heterohybridoma K6H6/B5. Idiotype was immobilized on 96-well polystyrene plates and exposed to $>10^{10}$ transducing units of bacteriophage libraries displaying N-terminal random peptides (8- or 12-mers) linked to the phage adsorption protein (pIII) (32). After washing away unbound phage, bound phage were eluted and

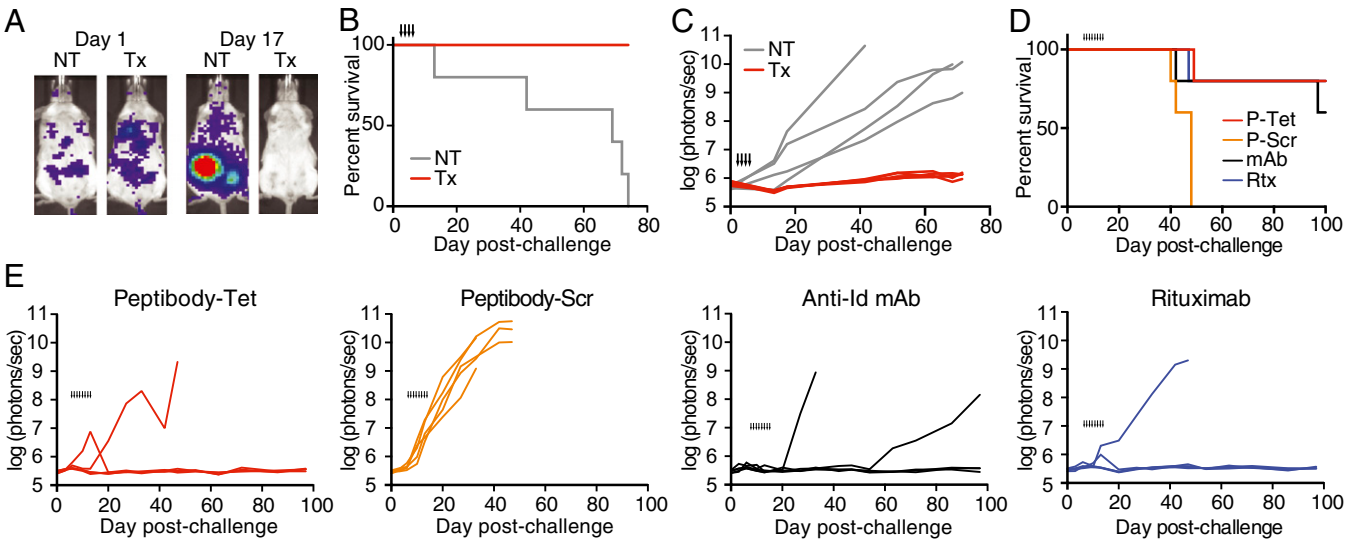

Fig. 5. Clearance of disseminated human lymphoma xenografts. (*A*–*C*) CB-17 SCID mice were challenged at day 0 with 1×10^6 SUPB8-Luc$^+$GFP$^+$ cells i.v. and, beginning on day 2, were treated once daily with i.p. injections of 65 μg of peptibody for 4 d. Tumor burden was quantified by near-infrared imaging (Fig. S4). (*A*) Representative images of untreated (NT) and treated (Tx) mice at two timepoints. (*B*) Survival comparison. (*C*) Tumor burden quantified by near-infrared imaging. (*D* and *E*) CB-17 SCID mice were challenged at day 0 with 1×10^6 SUPB8-Luc$^+$GFP$^+$ cells i.v. and, beginning on day 6, were treated once daily with i.p. injections of 50 μg of peptibody, scrambled peptibody, anti-idiotype mAb, or rituximab for 8 d. (*D*) Survival comparison. (*E*) Tumor burden quantified by near-infrared imaging.

amplified in K91 *Escherichia coli* cells for use in subsequent rounds of panning. After three rounds of panning, binding of individual phage clones to the tumor idiotype was confirmed by ELISA and the identity of the peptide expressed by each binding phage was determined by DNA sequencing. These sequences were aligned to identify a 9-mer consensus binding sequence (YXXEDLYRR). The optimal amino acid identity at degenerate locations in the consensus sequence (X) was queried by constructing a mutagenesis phagemid library where degenerate positions were left random and positions with known identity were varied but skewed toward the known consensus sequence with 70% encoding the "correct" identity and 30% with random identity. The final idiotype binding sequence identity (YSFEDLYRR) was determined by panning tumor idiotype with this mutagenesis library as described above.

Semisynthesis of Peptibodies. Peptide thioesters were produced by using 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase synthesis by a commercial vendor (CPC Scientific). To assemble a peptibody, the appropriate lyophilized peptide thioester was reconstituted at 1 mg/mL in distilled water and added to purified Fc protein in PBS pH 7.0 at an 8:1 molar ratio. MESNA (Sigma) was then added to a final concentration of 30 mM, and the reaction was allowed to proceed at room temperature for 48 h. A 100-fold molar excess of cysteine was then added to quench the ligation reaction and, 12 h later, the resultant peptibody was purified from the reaction mixture by Protein A affinity chromatography. The column was washed with PBS containing MESNA and cysteine, then with PBS alone. Peptibody was eluted with a pH 3.0 glycine buffer and brought to pH 7.0 with sodium-phosphate buffer.

Cell Lines. The SUPB8 human lymphoma line was previously derived from the bone marrow of a 15-y-old female with Burkitt lymphoma. To engineer SUPB8 GFP$^+$/Luc$^+$ cells, these cells were transfected with lentivirus containing an expression cassette with the firefly luciferase and GFP genes. Cells were sorted for GFP positivity to obtain stably transfected clones and iteratively subcloned to obtain cells expressing high levels of luciferase and GFP. The RAMOS human Burkitt lymphoma cell line was obtained from the American Type Culture Collection. Raji GFP$^+$/Luc$^+$ cells were a gift of the Irving Weissman laboratory (Stanford, CA).

Idiotype and Cell-Binding Assays. ELISA plates were coated with anti-human IgM polyclonal serum and used to adsorb soluble Ig from tumor cell rescue-hybridoma conditioned media. The peptibody or antibody was added at various concentrations, and binding was detected by anti-mouse IgG2a-HRP conjugated antibody. Absorbance was determined by using a Molecular Devices Spectramax Paradigm microplate reader. For flow cytometry-based assays, cells were exposed to various concentrations of peptibody or antibody, and binding was detected by fluorophore-conjugated anti-mouse IgG2a antibody. Cells were analyzed on a Becton Dickenson FACS Calibur flow cytometer.

Fluorescence Microscopy and Optical Sectioning. Cells were fixed with 1% paraformaldehyde and permeabilized with ice cold methanol, then stained with anti-human-IgM-PE and anti-mouse-IgG2a-FITC. Stained cells were imaged by using a Leica TCS SP8 confocal laser scanning microscope.

Caspase-3, Apoptosis, and in Vitro Viability Assays. For caspase-3 assays, cells were fixed with 1% paraformaldehyde and permeabilized with ice cold methanol, then stained with a PE-labeled antibody against cleaved human caspase-3 (BD Pharmingen). For apoptosis assays, cells were stained with PE-labeled Annexin-V (BD Pharmingen) and 7-AAD (Life Technologies). Cells were analyzed on a BD FACS Calibur flow cytometer. Viability assays were performed by adding the resazurin-based PrestoBlue cell viability reagent (Life Technologies) to wells of cell culture plates and measuring absorbance at 570 nm by using a Molecular Devices Spectramax Paradigm microplate reader; this value was compared with a standard curve to deduce the number of live cells per well.

Phagocytosis Assays. Mouse macrophages expressing RFP were cocultured with tumor cells expressing GFP and treated with the appropriate peptibody or antibody. After 24 h of coculture, cells were stained with propidium iodide to distinguish viable from nonviable cells and analyzed by using a Becton Dickinson LSR Fortessa flow cytometer. Phagocytosis was measured by the percentage of live GFP/RFP double-positive cells and, to account for differences in baseline propensity for phagocytosis between tumor cell lines, this percentage was normalized to the percentage of GFP/RFP double-positive cells.

Mouse Experiments. CB-17 severe combined immunodeficient mice (CB17/Icr-*Prkdc*^{scid}/IcrIcoCrl) at ∼8 wk of age were purchased from Charles River Laboratories. For therapy experiments, mice were injected with 1×10^6 SUPB8-Luc$^+$-GFP$^+$ cells at day 0. To quantify tumor burden, mice were injected i.p. with D-luciferin and imaged by using an IVIS 200 bioluminescence imaging system (Xenogen).

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