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Conservation of Pathogenic TCR Homology across Class II Restrictions in Anti-Ribonucleoprotein Autoimmunity: Extended Efficacy of T Cell Vaccine Therapy

YunJuan Zang,* Laisel Martinez,† Irina Fernandez,‡ Judith Pignac-Kobinger,* and Eric L. Greidinger*‡

T cells have been shown to mediate aspects of anti-ribonucleoprotein (RNP) autoimmunity, and are a potential target of therapy in lupus and related diseases. In this study, we assessed the relevance of a conserved class of anti-RNP T cells to autoimmune disease expression and therapy. Our data show that anti-RNP T cell selection induced a limited set of homologous CDR3 motifs at high frequency. Homologous CDR3 motifs have been reported in other autoimmune diseases. Vaccination with irradiated anti-RNP (but not anti–tetanus toxoid) CD4+ cells induced remission of anti-RNP–associated nephritis in 280% of treated mice, even with donor/recipient MHC class II mismatch, and in both induced and spontaneous autoimmune disease. Vaccine responder sera inhibited anti-70k T cell proliferation and bound hybridomas expressing the conserved CDR3 motifs. Our data indicate that a limited set of TCR CDR3 motifs may be important for the pathogenesis of anti-RNP lupus and other autoimmune diseases. The ability to target a consistent set of pathogenic T cells between individuals and across class II restrictions may allow for the more practical development of a standardized anti-RNP T cell vaccine preparation useful for multiple patients. The Journal of Immunology, 2014, 192: 4093–4102.

Autoimmunity to small nuclear ribonucleoprotein (snRNP) autoantigens occurs in systemic lupus erythematosus (SLE) and is particularly prevalent in early-onset and severe cases (1, 2). We have developed an induced murine model of anti-snRNP autoimmunity by immunizing mice with the 70kD subunit of the U1 snRNP (70k) along with the RNA component of this ribonucleoprotein (RNP), U1-RNA, a known TLR3 and TLR7 agonist (3, 4). This model shares clinical and immune features of human anti-snRNP autoimmunity, including spreading of the immune response from the 70kD subunit of the U1 snRNP (70k) to multiple other autoantigens, as well as the potential to induce nephritis (5, 6). Anti-RNP responses and nephritis (in the absence of anti-dsDNA or anti-chromatin Abs) have also been reported to develop spontaneously in the Trex1 deficiency model of SLE (7). These models provide opportunities to develop new therapeutic approaches to anti-RNP autoimmunity.

CD4+ T cells play an important role in anti-RNP autoimmunity and have previously been shown to be sufficient to induce RNP-associated antigenic spreading (8). Anti-RNP CD4+ T cells appear at high frequency (compared with healthy controls) in PBMCs in SLE patients and in the spleen and lesional tissues of anti-RNP autoimmune mice (9, 10). Adoptive transfer of anti-RNP–specific CD4+ T cells is sufficient to transfer nephritis in mice and to induce spreading autoimmune responses (6). Remarkably, TCR Vbeta CDR3 region motifs are conserved in the anti-RNP T cells we observe in multiple human patients and in our mouse model (9, 10). We therefore hypothesize that T cells expressing conserved CDR3 regions are pathogenic for anti-RNP autoimmunity and that T cell vaccination targeting this limited range of Ag-specific TCRs has the potential to be an effective immunotherapy.

Previous work has shown that treatments designed to target these anti-RNP T cells have been sufficient to induce significant clinical responses in both mice and humans (10, 11). However, these prior studies have not established that the beneficial effects observed were related to effects on pathogenic anti-RNP T cells as opposed to nonspecific immunomodulatory effects.

In this study, we establish that our previously reported protocol for vaccination with irradiated CD4+ T cells leads to clinical responses in established disease in a manner specific for vaccination with anti-70k T cells. We show that this vaccination induces T cell–targeted humoral responses that inhibit anti-70k T cell proliferation. We show that this vaccine therapy is effective across class II MHC restrictions and even in a model of spontaneous disease. These studies therefore suggest that a limited set of T cells play a key pathogenic role in anti-RNP autoimmunity even with diverse class II MHC (and may also mediate other non–RNP-associated autoimmune diseases), and that these T cells may be an important target for directed therapeutic interventions.

Materials and Methods

Animal studies

All mouse studies were performed following Institutional Animal Care and Use Committee–approved protocols. C57BL/6 mice were from The Jackson Laboratory. HLA-DR4tg mice, as previously described, were from...
Taconic. Female 8- to 10-wk-old mice were immunized s.c., as previously described (5, 6, 9, 10), with 50 μg each of 70k [as a maltose-binding protein (MBP) fusion protein (FP), 70k-FP] and U1-RNA together in 50 μl sterile PBS. For tetanus toxoid (TT) immunizations (performed concurrently on littermate female mice in the same cage as the anti-70k immunization recipients), 0.6 μg TT (GenWay Biotech, San Diego, CA) was administered s.c. in PBS. Successful immunizations were confirmed by 70k ELISA, as previously described (10, 12) and by TT commercial ELISA (GenWay), following the manufacturer’s instructions on serum samples from 1 mo after immunization. ELISA assays were performed in triplicate.

Trex1+/− STING+/− mice on a C57BL/6 background, as previously described, were a gift from Glen Barber (7). These were interbred for up to three generations, and the resulting genotypes of each mouse regarding Trex1 and STING was determined from tail clippings (Taconic, Cornova, TN). Mice were screened weekly for abnormal urinary sediment, beginning at 6 wk of age. Mice were entered into the study as matched pairs of anti-RNP+ (by anti-70k ELISA) littermate mice of the same sex, with the same Trex1 and STING genotype, and with parallel development of abnormal urine sediment. With each matched pair, one mouse was randomly assigned to receive anti-RNP T cell vaccination, and the other mouse to receive either PBS mock vaccination or anti-TT T cell vaccination. Animals were followed up clinically and assessed for kidney disease, as previously described (5, 6, 10). Briefly, urine dipsticks were assessed for protein and blood; serum was collected for creatinine levels and immune markers; and mice were sacrificed for blinded histological evaluation. At the time of sacrifice, spleen and lymph nodes were also obtained for further immune analyses.

T cell preparations
Ag-specific T cell lines were generated as previously described (6, 9, 10). Mice immunized with 70k + U1-RNA had T cells grown in the context of syngeneic naive APCs loaded with 70k; mice immunized with TT likewise had T cells grown with naïve syngeneic irradiated APCs loaded with TT. T cell proliferation assays were performed as previously described in the induction section below. Analyses of CDR3 sequences of 70k-selected T cells from three mice), whereas the I-Aβ germline B6 mice were more likely to have similar, but nonidentical, CDR3 sequences (in only one instance were two clones of the same sequence, CASRPGGLGQNTLYFG, identified).

We reviewed GenBank for all CD4+ T cells reported to have anti-CD4 T cell responses, without significant cross-reactivity on both backgrounds (Fig. 1). We sequenced the Vbeta CDR3 regions of 70k-selected and TT-selected T cell clones from the spleens and lesional tissues of 70k- and TT-immunized mice.

The 70k-selected T cells from 70k-immunized B6 and HLA-DR4tg mice had a high prevalence of TCR Vbeta CDR3 regions that were similar or identical to anti-70k T cells that we have previously identified from the spleen, lymph nodes, and lesional tissues of HLA-DR4tg mice and from the circulation of human patients with anti-70k autoimmunity (9, 10). Reanalysis of the prior sequences and the additional new sequences suggested that the consensus sequence CAS-G(Q/L)G-(E/T)Q(L)/Y(F)G provided the most robust definition of the shared Vbeta region. A significantly lower prevalence of T cells with V β regions that share this homology motif were identified among the TT-selected T cells compared with 70k-selected T cells on the HLA-DR4 background (Table I). Consistent with the reported role of HLA-DR4 as a risk factor for anti-RNP autoimmunity (15), the prevalence of 70k-specific, but not TT-specific, Vbeta CDR3 regions was higher in the HLA-DR4tg mice than the B6 mice. With the HLA-DR4 mouse in particular, we were more likely to identify multiple clones with identical Vbeta motifs (for example, the CASSEGGQRETLYG Vbeta CDR3 sequence was observed in 10 different anti-70k–selected clones from three mice), whereas the I-Aβ germline B6 mice were more likely to have similar, but nonidentical, CDR3 sequences (in only one instance were two clones of the same sequence, CASRPGGLGQNTLYFG, identified).

We sequenced 70k-selected T cells from three mice, and the resulting genotypes of each mouse regarding CDR3 were characterized by their expression of CDR3 sequences in this study. The majority of the human sequences were from patients with autoimmune or allergic disease (Supplemental Table I; 12 of 23, 52%), most
frequently patients with multiple sclerosis (MS), rheumatoid arthritis (RA), or lupus/connective tissue disease (CTD). Furthermore, 21 of 25 (84%) of the previously reported murine sequences were from mice with autoimmune disease, including most prominently lupus/CTD but also experimental allergic encephalomyelitis (an MS model), celiac disease, collagen-induced arthritis (an RA model), and type 1 diabetes (T1D) among others. Thus, this Vbeta CDR3 motif appears to be prominently represented in the setting of dysregulated immunity.

When we used more permissive standards for defining the Vbeta CDR3 homology region in our Ag-selected CD4+ T cells (up to three mismatched residues versus the homology motif), we identified 46 additional human CD4+ T cell Genbank sequences, 29 additional murine CD4+ T cell Genbank sequences, and 30 additional sequences from cells isolated for this report (Supplemental Table II). Immune dysregulation diseases were present in 29 of 46 (63%) human subjects, including prominently SLE/CTD, RA, and MS patients; 16/29 murine samples (55%) had autoimmune diseases including SLE/CTD, experimental allergic encephalomyelitis, and T1D. In contrast, none of the non-disease states were also associated with this motif, including human T cells with TT peptide reactivity, one of which was selected on an HLA-DR4 background.

However, we found that the substantial difference in the frequency of the homology motif between HLA-DR4tg mice for 70k versus TT-selected T cells became even more pronounced using the more permissive inclusion criteria (Table I, Fisher’s exact, \( p < 0.0001 \)). Similarly, the difference in the frequency of 70k-selected T cell sequences derived from HLA-DR4 versus B6 mice also became even more pronounced (Fisher’s exact, \( p = 0.0002 \)).

We next considered the Valpha CDR3 regions in our T cells. In HLA-DR4tg mice, we identified a Valpha CDR3 shared homology motif for 70k-selected T cells, CA(A/V)(S/R)–GGS–(K/R)–L–FG, in 18% of the sequences evaluated (Table II). When CDR3 Valpha regions from our 70k-selected CD4+ T cells from HLA-DR4tg mice were allowed to have up to two mismatched residues, 64% of the Valpha regions we isolated had homology to this motif. To ensure that this rate of Valpha CDR3 homology was distinct from what would be expected from an arbitrary population of non-autoimmune TCR Valpha regions, we compared our homology rate with that seen in a set of CDR3 Valpha regions from the leukemic cells from a recently published cohort of Sézary syndrome patients (16). None of the 13 Valpha sequences from this cohort had complete homology with our Valpha motif, and only 2 of the Valpha sequences from this cohort differed for our consensus motif by ≤2 residues (2 of 13 versus 28 of 44 of our anti-70k T cell Vα sequences, Fisher’s exact, \( p = 0.004 \)).

A GenBank search of human and murine Valpha sequences from CD4+ T cells revealed 5 human and 14 murine CDR3alphas that were found to have complete homology to our Valpha motif. Among the human cases, this included one T cell clone each from an RA patient (AAA61064) and an MS patient (AAB32430.1). The murine homologous CDR3alphas were from NOD anti-insulin B peptide 9-23 T cell clones in 10 of the 14 cases identified (17), and also from an anti-insulin BALB/C T cell clone in an 11th case (AAA40345). One of the NOD T cell clones (12-4.4) shared complete homology with both our anti-70k CDR3 Valpha and CDR3 Vbeta motifs (18).

One GenBank search from an anti-TT peptide-specific human T cell showed complete identity to our Valpha homology motif (19), but the Vbeta CDR3 region from this clone differed from our homology motif at 5 of the 11 defined residues. This clone was reported to respond to the TT peptide presented by HLA-Dw6b, but not by HLA-DR4 (19). This evidence supports the hypothesis that

Table I. Frequency of conserved TCRβ CDR3 sequence in Ag-selected splenic CD4+ cells from DR4 and B6 mice

<table>
<thead>
<tr>
<th>Conserved CDR3</th>
<th>Frequency</th>
<th>Fisher’s Exact ( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS–G(L/Q)G–(E/T)(Q/L)G(Y/F)FG</td>
<td>DR4-70K</td>
<td>B6-70K</td>
</tr>
<tr>
<td>Stringent criteria (complete match)</td>
<td>21.2% (35/165)</td>
<td>7.9% (11/139)</td>
</tr>
<tr>
<td>Permissive criteria (≤3 mismatches)</td>
<td>40.0% (66/165)</td>
<td>20.1% (28/139)</td>
</tr>
</tbody>
</table>

DR4 = HLA-DR4tg B6 mice without any endogenous class II expression; B6 = germline C57BL/6 expressing homologous I-α2; 70k = T cells selected with the U1-70kD snRNP autoantigen from mice immunized with the same Ag; TT = T cells selected with TT from mice immunized with the same Ag. The frequencies of TCRs with the consensus CDR3 region described was calculated for TT- and 70k-selected cells from TT- or 70k-immunized mice.
anti-70k and anti-TT T cells have structurally distinct CDR3 regions when both the Valpha and Vbeta chains are considered.

When we screened a series of T cell hybridomas generated concurrently from 70k-selected CD4+ T cells (derived from mice with anti-RNP autoimmunity) for reactivity to 70k presented by syngeneic naive APCs, we found that the presence of the Vbeta and Valpha conserved motifs was associated with increased anti-70k responses (Fig. 2). These results show that the shared a and b CDR3 regions observed in 70k-selected T cells can participate together in anti-70k recognition in anti-RNP autoimmunity. The results also suggest that T cell CDR3 regions with less than complete homology to our consensus motif could also contribute to anti-70k responses.

Efficacy of T cell vaccine therapy for anti-RNP nephritis is Ag but not MHC dependent

To determine whether the benefit of CD4+ T cell vaccination that we have previously reported for anti-RNP nephritis was due to a nonpecific response to irradiated CD4+ T cell debris, as opposed to an Ag-specific response, we repeated T cell vaccine studies on mice with nephritis due to induced anti-RNP autoimmunity. Instead of PBS, as in our previous report (10), the control group received the same dose of irradiated Ag-selected CD4+ T cells as the treatment group, except the CD4+ T cells in the control group were from TT-immunized mice selected in vitro with TT (rather than from 70k-immunized mice selected in vitro with 70k for the treatment group).

We observed that with HLA-DR4tg mice, consistent with our prior report, active urinary sediment had resolved 2 mo after treatment in five of five mice receiving anti-70k irradiated T cells, but in none of five mice receiving anti-TT irradiated T cells (Fisher’s exact = 0.008) (Fig. 3). These results demonstrate that the beneficial effect of the irradiated T cell exposure was not due to a nonspecific effect mediated by irradiated T cell debris. Because some anti-TT–specific T cells express the conserved Vbeta CDR3 region, the failure of anti-TT–selected T cells to induce a protective response suggests that this Vbeta CDR3 region is unlikely to be sufficient to induce protective immunity in responder mice.

We have previously observed that immunization of germline B6 mice with 70k + U1-RNA induces anti-RNP autoimmunity and end organ manifestations similar to what we have reported with HLA-DR4tg mice (in which native class II expression has been knocked out) (6, 10). We therefore assessed the ability of germline B6-

### Table II. Vα CDR3 sequences in 70k-selected CD4+ T cells

<table>
<thead>
<tr>
<th>CDR3 Sequence</th>
<th>MHC Restriction</th>
<th>Accession No.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous to consensus motif</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAASGGSALRGLHFG</td>
<td>HLA-DR4</td>
<td>KC202828</td>
<td>4 clones</td>
</tr>
<tr>
<td>CAASDQGGSALKLHFG</td>
<td>HLA-DR4</td>
<td>KC202844</td>
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<td>CAVRDGGSNKLYLTFG</td>
<td>HLA-DR4</td>
<td>KC202846</td>
<td>4 clones</td>
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<td>CAVRSGGSNKLYLTFG</td>
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<td>KC202852</td>
<td></td>
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<td>CAVSDDGGSNKLYLTFG</td>
<td>HLA-DR4</td>
<td>KC202853</td>
<td></td>
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<td>CAASNNGGSALRGLHFG</td>
<td>HLA-DR4</td>
<td>KC202864</td>
<td></td>
</tr>
<tr>
<td>Near homology to consensus motif (±2 residues off)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CAASGSSNNRIFFG</td>
<td>HLA-DR4</td>
<td>KC202827</td>
<td>2 clones</td>
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<td>CAASGTGADRLTIFG</td>
<td>HLA-DR4</td>
<td>KC202830</td>
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<td>CAASGDGSKNLKLFH</td>
<td>HLA-DR4</td>
<td>KC202832</td>
<td></td>
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<td>CAVRSGGAGYKYYKVF</td>
<td>HLA-DR4</td>
<td>KC202834</td>
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<tr>
<td>CAVSGTGGYKYYKVF</td>
<td>HLA-DR4</td>
<td>KC202838</td>
<td>4 clones</td>
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<td>2 clones</td>
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<td>CAVSAGGALGF</td>
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<td>Other sequences identified</td>
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<td>HLA-DR4</td>
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<td>HLA-DR4</td>
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<td>KC202851</td>
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<td>CVLDDFGNKLIIWG</td>
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<td>KC202856</td>
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<td>CAVSEDNNAPRLF</td>
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<td>KC202858</td>
<td>9 clones</td>
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<tr>
<td>CAVRNVNNLVLHFH</td>
<td>HLA-DR4</td>
<td>KC202863</td>
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</table>
derived anti-70k CD4+ irradiated T cell vaccine to treat nephritis in B6 mice with induced anti-RNP autoimmunity. We also assessed the ability of the HLA-DR4tg vaccine to treat germ-line B6 mice with induced anti-RNP autoimmune nephritis. TT-specific T cell vaccines on the B6 and HLA-DR4tg backgrounds were used as control stimuli.

In these experiments, we observed that the anti-TT–specific irradiated CD4+ T cells had no impact on disease (persistent nephritis in five of five B6 mice in both the B6 and HLA-DR4tg irradiated T cell recipient groups) (Fig. 3). However, with the 70k-specific T cell vaccinations, remission of active urinary sediment was observed in four of five B6 mice in both the B6 and HLA-

![FIGURE 2](http://www.jimmunol.org/)

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**FIGURE 2.** Conserved CDR3 Valpha and Vbeta regions are associated with anti-70k reactivity. IL-2 secretion by mouse T cell hybridomas expressing anti-RNP TCRs after 48-h stimulation by irradiated syngeneic APCs loaded with 70k versus control stimuli (MBP) was measured by commercial ELISA. Anti-70k reactivity above baseline was not seen in hybridomas lacking the majority of the Vbeta homology motif (Hyb 9, Hyb 11). The amount of IL-2 production in Hyb 50, Hyb 114, and Hyb 133 appears to correlate with the extent of homology with the conserved Vbeta and Valpha CDR3 motifs.

---

**FIGURE 3.** Efficacy of MHC-mismatched, but not Ag-discrepent, T cell vaccination in nephritic anti-RNP mice. With use of anti-70k–immunized mice on either the HLA-DR4tg or the B6 background, urine was assessed prior to (pretreatment) and 2 mo after (posttreatment) a single s.c. instillation of irradiated anti–U1-70k–specific T cells (anti-70k T cell vax) or irradiated anti-TT T cells (anti-TT T cell vax). T cell vaccines were prepared on the HLA-DR4tg or B6 background and administered to immunized mice with either the same (matched) or opposite (mismatched) class II motifs. Mice with proteinuria were characterized as having nephritis. At 2 mo posttreatment, urine was again assessed and results were confirmed by renal histology. The anti-70k T cell vaccines reduced the rate of nephritis dramatically in both MHC-matched and -mismatched conditions, whereas the anti-TT T cell vaccine had no benefit for nephritis in either matched or mismatched mice. *Fisher’s exact, p = 0.048 versus pretreatment; **Fisher’s exact, p = 0.008 versus pretreatment.
DR4tg vaccine recipient groups (Fisher’s exact, \( p = 0.048 \)). Thus, the ability to respond to anti-70k T cell vaccination was not limited by class II differences.

**Induction of inhibitory anti-T cell Abs by T cell vaccination**

To assess potential mechanism(s) of action of the T cell vaccine therapy, we screened the sera of HLA-DR4tg recipients of HLA-DR4tg–derived anti-70k–selected T cells for their ability to inhibit T cell proliferation via humoral factors (Fig. 4A). Sera from the five HLA-DR4 mice (from Fig. 3) immunized with 70k-FP who developed proteinuria that disappeared after they received matched anti-70k–selected irradiated T cell vaccine (responder mouse sera) were tested. Splenocytes from 70k-immunized HLA-DR4tg mice proliferated as expected in response either to 70k-FP itself or to a stimulatory anti-CD3 Ab in the presence of a normal control murine serum. Two distinct patterns were observed with individual responder sera in the context of anti-CD3 stimulation. In some cases, anti-CD3–induced proliferation was inhibited (responders 1, 2, and 5), whereas in others anti-CD3–induced inhibition was unaffected or even potentiated (responders 3 and 4). The cases in which anti-CD3–induced proliferation was inhibited by responder serum also showed a consistent trend toward inhibition of 70k-induced proliferation as well (\( t \) test versus control serum, \( p = 0.03, 0.05, \) and 0.07 for responders 1, 2, and 5, respectively). The two responder sera that did not inhibit anti-CD3 proliferation showed different patterns of effects on Ag-specific proliferation. Responder 3 appeared to potentiate anti-MBP responses and to have no effect on anti-70k responses. Responder 4 appeared to have no effect on anti-MBP responses and to show a trend toward reducing anti-70k responses (\( t \) test versus serum control, \( p = 0.1 \)).

**FIGURE 4.** Anti-T cell humoral responses are induced by anti-70k T cell vaccination. (A) Sera from anti-70k T cell vaccination responders inhibit proliferation of splenocytes from 70k-immunized mice. 70k-immunized HLA-DR4tg splenocytes were incubated alone or with one of the following stimuli: 70k-MBP fusion construct (70kD-FP) used to immunize the mice (25 \( \mu \)g/ml), 20 \( \mu \)g/ml of MBP alone, or anti-mouse CD3 Ab at a concentration (10 \( \mu \)g/ml) that yielded similar proliferation levels. Incubations were for 48 h in vitro in complete medium in the presence of equal quantities of either normal murine serum or responder murine sera from HLA-DR4tg mice treated with anti-70kT cell vaccine from HLA-DR4tg donors. Cells were pulsed with tritiated thymidine for the final 18 h in culture, then harvested; cpm of incorporated tritium were measured (see Materials and Methods). All assays were performed in triplicate. Results are reported as fold tritiated thymidine incorporation relative to unstimulated cells. (B) Responder sera IgG recognize anti-70k TCR expressing hybridoma cells. Hybridoma cells from Fig. 2 were incubated with anti-CD3 IgG or equal dilutions of the indicated sera, washed, incubated with a fluorescence-labeled goat anti-mouse IgG secondary Ab, and counted by flow cytometry (10,000 cells counted per condition). Gating was set based on normal serum control results with all three hybridomas, and held constant for all conditions.
Consistent with these two patterns of T cell inhibition, we observed two patterns of IgG binding by responder sera to T cell hybridomas, as measured by flow cytometry (Fig. 4B). Responder 1 serum, which inhibited both anti-CD3- and 70k-induced proliferation, showed promiscuous IgG binding to hybridomas regardless of the degree of similarity of the TCR Vbeta and Valpha chains to the anti-70k homology motifs. In contrast, responder 4 serum, which appeared to selectively inhibit only 70k-induced proliferation, showed stringent IgG recognition only of the HbB-133 line, with complete representation of both the Valpha and Vbeta homology motifs. The selective recognition of HbB-133 by responder 4 serum is particularly striking because the anti-CD3 staining of this hybridoma was low, suggesting relatively low surface expression of intact TCRs in this cell line. The high recognition by responder 1 serum of all three tested hybridomas, despite variable levels of anti-CD3 recognition, suggests that non-TCR structures may also be targeted in some cases by effective humoral responses to anti-70k T cell vaccination. The lack of T cell inhibition by responder 3 serum suggests that mechanisms other than, or in addition to, humoral inhibitory effects can also mediate the clinical benefit of anti-70k T cell vaccination in some cases.

Anti-RNP T cell vaccination in a spontaneous disease model

To assess the impact of anti-RNP T cell vaccination (using T cell vaccine developed in HLA-DR4tg mice) in a spontaneous model of lupus-like autoimmunity with nephritis, we used Trex1-deficient mice. Of 11 Trex1−/− mice screened (all of which had at least one STING+ allele), 3 (27%) developed abnormal urinary sediment by 12 wk of age, and no genetically matched pair of mice existed to be entered into the study, and none were anti-RNP+. Of 31 Trex1−/− mice with at least one STING+ allele, 30 (97%) had abnormal urinary sediment (Fisher’s exact versus Trex1+/− mice, p < 0.0001), 24 could be allocated in matched pairs, and 21 of 24 were anti-RNP+ by ELISA (88%), leading to 10 matched pairs of mice that entered the T cell vaccination study. Of three Trex1−/− mice that were STING−/−, two had abnormal urinary sediment, were a matched pair genetically, both were anti-RNP+, and thus entered the study as an additional pair. Of 12 Trex1−/− mice, 11 (92%) had abnormal urinary sediment (p = 0.003 versus Trex1+/− mice). This included the one Trex1−/+ mouse that was STING−/−. A total of four matched pairs of Trex1−/− mice, all with at least one STING+ allele, and all anti-RNP+, were thus also entered into the study.

Thus, a total of 14 total pairs of matched anti-RNP+ Trex1−/− (Trex1−/− or Trex1−/+ mice) with renal disease were studied. The first seven of the study mouse pairs were randomly assigned to anti-RNP T cell vaccine (made from HLA-DR4tg mice) versus PBS mock treatment (Fig. 5A). Normalization of urinary sediment was noted in six of seven (86%) T cell vaccine-treated mice, but in only one of seven (14%) PBS mock-treated mice (Fisher’s exact, p = 0.03). The next seven study mouse pairs were randomly assigned to anti-RNP T cell vaccine or anti-TT T cell vaccine (both made from HLA-DR4tg mice) (Fig. 5A). Identical to the first set of studies, normalization of urinary sediment in mice treated with anti-RNP T cell vaccine recipients, but in only one of seven (14%) anti-TT T cell vaccine recipients (p = 0.03). The time to normalization of the urinary sediment after anti-RNP T cell vaccination was 2–4 wk (mean, 3.0 ± 0.6 wk). Consistent with previously reported results in the induced anti-RNP model, anti-RNP IgG levels by ELISA were not reduced in anti-RNP T cell vaccine–treated mice, and did not differ between these and control mice (data not shown).

Proliferation studies on whole spleen cells were performed concurrently on mice treated with anti-RNP T cell vaccine (n = 9) and either PBS mock-treated (n = 4) or TT T cell vaccine (n = 5) matched controls in the presence of RNP peptide (70k), antimusrine CD3 Ab (α-mCD3), or medium only (Fig. 5B). With 70k peptide stimulation, proliferation was suppressed in the anti-RNP T cell vaccine–treated group compared with both the PBS mock-treated (t test, p = 0.003) and TT T cell vaccine (t test, p = 0.006) groups. No differences between the anti-RNP T cell vaccine–treated mice and either the PBS or anti-TT T cell vaccine groups could be identified in anti-CD3 or medium-only conditions, although trends toward similarly reduced proliferation to anti-CD3 stimulation with both the anti-RNP and anti-TT T cell vaccine groups, compared with the PBS mock-treated group, were noted. Improvement in clinical and immune parameters was paralleled by improvements in the histological appearance of anti-RNP T cell vaccine–treated compared with control kidneys (Fig. 5C).

Of the Trex1−/− mice studied, two matched pairs consisted of STING−/+ mice. Both of these pairs were in the anti-RNP versus anti-TT study arm. Myocarditis and early death (at 16 wk) were observed in both Trex1−/− STING−/+ mice that received anti-TT T cell vaccine. Both of these mice had persistently abnormal urine sediment. In contrast, both of the Trex1−/− STING−/+ mice that received the anti-RNP T cell vaccine showed normalization of their urine sediment. One of these two mice, however, also died with myocarditis at 16 wk of age. Neither myocarditis nor early death was observed in any of the Trex1−/+ mice in our study. We also did not detect myocarditis and had no cases of early death in any of the four Trex1−/− STING−/+ mice in our study.

Discussion

Anti-RNP autoimmunity in humans occurs in a subset of SLE patients at increased risk for severe disease (1, 2). We have previously shown that adoptive transfer of 70k-specific T cells is sufficient to induce nephritis in a murine model (6). T cell vaccine immunotherapy has the potential to reduce the impact of disease in patients with anti-RNP autoimmunity without exposing them to the risks of the more nonspecific immunosuppression that characterizes currently available clinical treatment options. The studies reported in this article provide evidence that targeted immunotherapy in anti-RNP autoimmunity can have excellent immunological and clinical efficacy. We show that anti-70k T cell vaccination therapy can induce Abs that inhibit Ag-specific T cell proliferation and can reverse established end organ disease, even across class II MHC restrictions, and even in spontaneous disease.

Our analysis of conserved Vbeta and Valpha CDR3 regions revealed frequent homologous Valpha or Vbeta regions in both humans and mice with anti-RNP autoimmunity, despite differences in class II MHC. The same CDR3 motifs have been previously reported in the presence of autoimmune diseases (most notably in the presence of human and animal models of MS, RA, and T1D). Either the Valpha or Vbeta motif has also been reported on T cells in the absence of autoimmune disease, but to date the combination of both the conserved Valpha and Vbeta region in the same T cells has been reported only with T cells from autoimmune disease subjects. Analysis of such a clone with complete conservation of both the Valpha and Vbeta CDR3 regions has been reported (17, 18), showing that this T cell clone is diabetogenic upon adoptive transfer in NOD mice and suggesting that a modified insulin peptide binds in register 3 of the class II molecule to provide an antigenic stimulus to this TCR. We similarly have reported that our anti-70k T cells mediate renal disease and anti-RNP autoimmunity on the HLA-DR4 background (6).

Given their presence in non–disease subject T cells, these Valpha and Vbeta CDR3 motifs must also arise in normal indi-
viduals. A predilection for T cells expressing such TCRs in the peripheral circulation or tissues may be one factor that predisposes to autoimmune responses. It remains to be determined whether vaccination therapy targeting these TCR motifs would leave a clinically significant “hole” in protective T cell immunity.

**FIGURE 5.** Response to anti-RNP T cell vaccine in a spontaneous disease model. Matched pairs of Trex1-deficient mice with anti-RNP autoimmunity and nephritis were treated with anti-RNP T cell vaccine or with either PBS mock vaccination or anti-TT control vaccine as in Fig. 3 within 2 wk of the onset of active urinary sediment (onset of active urinary sediment, including proteinuria, noted at between 8 and 12 wk of age in all study mice). Urine was then assessed weekly, and mice were sacrificed 1 mo later. (A) Clinical resolution of active urine sediment was observed in 86% of anti-RNP T cell vaccine–treated mice in both groups. Both groups of control mice had spontaneous resolution of urine sediment in only 14% of cases (*Fisher’s exact, p = 0.03). (B) Cellular response to T cell vaccination. Proliferative responses to 70k peptide were assessed by tritiated thymidine incorporation (in cpm) of whole splenocytes cultured with no specific stimulus (medium), with the addition of RNP peptide Ag (70k-FP), or with anti-murine CD3 (α-mCD3) in cell preparations from study mice treated with anti-RNP T cell vaccine (anti-RNP TCV) or their matched controls (PBS mock-treated or anti-TT T cell vaccine recipients [anti-TT TCV]). Anti-RNP T cell vaccine induced significant inhibition specifically of 70k peptide–induced proliferation, compared with both PBS-treated (*t test, p = 0.003) and anti-TT vaccine–treated conditions (*p = 0.006). (C) Histological response to T cell vaccine therapy. Representative images of H&E-stained sections (×10 magnification, same exposure, equally cropped) from a matched set of study mice (Trex1+/− STING+/+) show glomerular hypercellularity in the PBS control mock-treated kidney, but not in the anti-RNP T cell vaccine–treated kidney.
lection did not have an impact on clinical anti-RNP autoimmunity when delivered as a component of our anti-TT T cell vaccine, autoimmune TCR responses (at least to 70k) may depend on the presence of the Valpha CDR3 homology motif in addition to the Vbeta CDR3 region.

The frequent identification of homologous CDR3 motifs from other autoimmune disease T cells raises the question of whether such a therapeutic vaccine might also have efficacy against MS, RA, or T1D. The homology of anti-RNP pathogenic T cells across MHC restrictions (and potentially across disease processes) also brings up the question of whether atypical modes of Ag presentation may contribute to this conservation of TCR motifs, and potentially play a role in the emergence of pathogenic T cells in autoimmunity in the settings of anti-RNP lupus, as well as MS, RA, and T1D (20, 21).

Although anti-RNP responses have previously been indirectly appreciated in Trex-deficient mice, in this report we identify both anti-RNP immune responses and nephritis in Trex<sup>−/−</sup> and Trex<sup>−/+</sup>-STING-deficient mice, conditions in which myocarditis was not observed. Further studies are needed to dissect the relationships between Trex deficiency, innate immunity, and the specificity of autoimmune targeting. Trex alleles conferring increased lupus risk have been previously linked to anti-RNP responses in human cohorts (22). The success of anti-RNP T cell vaccination in this system need not imply that this strategy will be similarly effective in human lupus, but it is notable that trials with p140/Lupuzor, a T cell tolerogen structurally related to the 70k peptide of the U1-70kDa small nuclear ribonucleoprotein (snRNP), has shown promising results (11, 19, 20).

Limitations of this study include the relatively small number of mouse strains included. In the absence of studies on anti-TT (or other control) hybridomas, we cannot conclude that the patterns of serum additionally suggests that vaccine therapy can elicit relevant immunomodulatory effects mediated by cellular immunity. Future studies will be needed to more broadly characterize the immune responses that anti-70k T cell vaccine responders sera binding are specific for anti-RNP targets may in some cases contribute to the immunomodulatory effects. The lack of T cell inhibition in vitro with responder 3 serum additionally suggests that vaccine therapy can elicit relevant immunomodulatory effects mediated by cellular immunity. Future studies will be needed to more broadly characterize the immune responses that anti-70k T cell vaccine responders develop. The ability of a T cell vaccine to show benefit across MHC restrictions suggests that uniform T cell vaccine production (rather than the need for personalized vaccines developed for each individual patient) may be an effective strategy that could reduce the expense and delay in providing potentially effective T cell vaccine therapy to patients with autoimmune disease.

This report provides new evidence to support the critical role of T cells in anti-RNP lupus, by demonstrating that an anti-RNP–specific T cell vaccine can have therapeutic benefit in an Ag-specific manner. Moreover, our findings highlight a subset of TCR CDR3 motifs as potentially relevant to the pathogenesis of both anti-RNP autoimmunity and other major autoimmune diseases.

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References


