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Evidence for Molecular Mimicry between Human T Cell Epitopes in Rotavirus and Pancreatic Islet Autoantigens

Margo C. Honeyman,* Natalie L. Stone,* Ben A. Falk, † Gerald Nepom, † and Leonard C. Harrison*

In type 1 diabetes, autoreactive T cells mediate destruction of insulin-producing pancreatic β cells. The major genetic contribution to the lifetime risk of type 1 diabetes comes from HLA genes in the MHC that encode proteins that present antigenic peptides to T cells (1). Several lines of evidence demonstrate that genetic susceptibility to type 1 diabetes is strongly modified by environmental factors. Thus, the disease is discordant in identical twins (2), its incidence has risen progressively over the last 40 y, especially in younger children (3) concomitant with a decreased contribution of high-risk HLA genes (4), and circumstantial evidence implicates environmental agents including viruses (5).

Circulating autoantibody markers of pancreatic islet autoimmunity are directed against four defined autoantigens: (pro)insulin, the Mr 65,000 isoform of glutamic acid decarboxylase (GAD65), tyrosine phosphatase-like insulinoma Ag 2 (IA2) (6), and zinc transporter 8 (7). In at-risk children followed from birth in the Australian BabyDiab Study, we documented a temporal association between rotavirus (RV) infection and the first appearance of, or an increase in, insulin autoantibodies and autoantibodies to GAD65 or IA2 (8).

RVs, dsRNA viruses of the family Reoviridae, are the major cause of gastroenteritis in infants worldwide, being transmitted by fecal-oral contamination and activated in the small intestine by pancreas-derived trypsin. RV infection has been associated with pancreatitis (9–11), and we demonstrated that RV infects β cells (12).

We observed that the strongly immunogenic VP7 protein of RV contains a peptide sequence (aa40–52) highly similar to one in IA2 (aa805–817), as well as a contiguous sequence (aa16–28) highly similar to one in GAD65 (aa115–128) (13) (Fig. 1). Both the IA2 and GAD65 sequences are T cell epitopes restricted by HLA-DR4 (DRB1*0401) (14). Subsequently, the IA2 epitope was shown to be processed naturally by APCs in vitro (15). The GAD65 sequence was also found to be a CD4+ T cell epitope in HLA-DRB1*0401 transgenic mice (16, 17). Recently, these IA2 and GAD65 sequences were shown to encompass dominant HLA-A2–restricted epitopes for CD8+ T cells in individuals with recent-onset type 1 diabetes (18, 19). Collectively, these findings support the hypothesis that molecular mimicry between immunogenic peptides in RV-VP7 and similar peptides in IA2 and GAD65 is a mechanism by which islet autoimmunity may be triggered or exacerbated. To obtain further evidence for this hypothesis, we first determined if the similar RV-VP7 peptide could stimulate T cells expanded to the IA2 epitope (19). To identify T cell epitopes restricted by type 1 diabetes-associated HLA haplotypes, heparinized venous blood was obtained from three individuals who were homozygous for HLA-DRB1*0301-DQB1*0201 (two at risk for type 1 diabetes, one healthy) and from three individuals homozygous for HLA-DRB1*0401-DQ8 (two at risk for type 1 diabetes, one healthy). The individuals at risk had Abs to the islet Ags GAD65 and IA2 and were pre-diabetic, developing clinical type 1 diabetes within 2 y of testing. To confirm...

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Abbreviations used in this paper: CBV, Coxsackie B virus; GAD65, glutamic acid decarboxylase; IA2, insulinoma Ag 2; RV, rotavirus.

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Materials and Methods

Subjects

To identify T cell epitopes restricted by type 1 diabetes–associated HLA haplotypes, heparinized venous blood was obtained from three individuals who were homozygous for HLA-DRB1*0301-DQB1*0201 (two at risk for type 1 diabetes, one healthy) and from three individuals homozygous for HLA-DRB1*0401-DQ8 (two at risk for type 1 diabetes, one healthy). The individuals at risk had Abs to the islet Ags GAD65 and IA2 and were pre-diabetic, developing clinical type 1 diabetes within 2 y of testing. To confirm...
these epitopes in a range of subjects at risk for type 1 diabetes, blood was obtained from 52 predominantly HLA-heterozygous islet autotransplant-positive, first-degree relatives of people with type 1 diabetes (27 males, 25 females; median age 12 y, range 4–57 y) and from 27 of their islet autotransplant-negative healthy siblings (11 males, 16 females; median age 12 y, range 5–55 y). HLA phenotypes for these subjects are shown in Table II.

To determine if T cells expanded to the IA2 epitope would respond by expression of the proinflammatory cytokine IFN-γ after restimulation by the similar RV-VP7 peptide, blood was obtained from a 19-year-old at risk female with Abs to GAD65 and IA2 and the highest-risk HLA genotype (A1;2, B8;44, DRB1*0301,*0401,DQ2,8). To determine if CD4+ T cells cloned to the IA2-like RV-VP7 peptide could recognize the IA2 epitope, blood was obtained from a 21-year-old male with the intermediate-risk HLA genotype (A2,25, B39;44, DRB1*0401,*1501,DQ1,8) and autotransplant-negative healthy siblings, IA2 and GAD65.

All blood samples were collected between 8:30 and 10:00 AM. The study was approved by the Melbourne Health Human Research Ethics Committee and conducted with informed consent.

HLA typing

Subjects were typed for type 1 diabetes susceptibility HLA class II haplotypes DR4-DQ8 and DR3-DQ2 by sequence-specific oligotyping, following the International Histocompatibility Workshop protocol. HLA class I alleles were typed by the standard microlymphocytotoxicity method for all recognized alleles.

Islet autoantibody assays

Abs to islet Ags were measured in internationally standardized assays (e.g., see Ref. 20), by immunoprecipitation of [35S]methionine-labeled recombinant IA2 or GAD65 or by precipitation of [125I]insulin.

Peptides

Previously defined T cell epitope peptides in IA2 805–817 (VIVMLT-PLVEDVG) and GAD65 115–127 (MNILLQYYVKSFD) and the similar sequences in VP7 of the human G RV-VP7 strain P) were measured by a direct-competition ELISA as previously described (22). The binding affinities of IA2 805–817, GAD65 115–127, and RV-VP7 40–52 peptide. PBMCs at 105/ml in PBS were labeled with 0.2 μM of the dye 5, CFSE for 5 min at 37°C, and washed with PBS containing first 1% and then 0.1% pooled human serum. Cells in IMDM (Life Technologies) containing 5% pooled human serum, 100 mM nonessential amino acids, 2 mM glutamine, and 5 × 10−3 M 2-ME (complete IMDM) were placed at 2 × 105/well into 20 replicate U-bottom wells of a 96-well plate, with 25 μg/ml final concentration of IA2 805–817, PBS carrier alone (negative control), or 5 LFU/ml preservative-free tetanus toxoid (CSTL, Melbourne, Australia) (positive control) and incubated at 5% CO2 in air at 37°C for 7 d. At this time, thawed autologous PBMCs were labeled with double-strength CFSE to be used as APCs, double labeling allowing discrimination by flow cytometry from previously labeled cells that had not divided. Cells from the 7-d cultures were washed, and 6 × 104 cells in 5 ml complete IMDM were added per well to a 12-well plate (Corning Life Sciences, Corning, NY) containing 6 × 105 APCs/well with either 25 mg/ml IA2 805–817 or RV-VP7 40–52 peptide. After 24 h in 5% CO2 in air at 37°C, 2 μl well GolgiStop from the BD Cytofix/Cytoperm kit (BD Biosciences, San Diego, CA) was added and the cells incubated a further 4 h. A total of 1 μl/mouse propidium iodide was added to detect cell death, the cells washed twice in PBS with 0.1% BSA and 0.02% EDTA at 4°C, stained with APC-conjugated anti-CD4 (RPA-T4; BD Pharmingen, San Diego, CA), vortexed briefly and incubated for 20 min on ice protected from light, and then washed twice in PBS/BSA/EDTA. Cells were fixed and permeabilized with 250 μl/tube Cytofix/Cytoperm solution (BD Biosciences), vortexed briefly, and incubated at 4°C for 20 min. After two washes in 1% Permwash solution diluted in sterile water, cells were stained with PE-conjugated mouse anti-human IFN-γ (NIB42; BD Pharmingen) or mouse PE-IgG1 as isotype control (MOPC-21, BD Pharmingen). After two washes with 1% Permwash, cells were resuspended in 300 μl PBS and 0.1% BSA, and stored at 4°C in the dark. Four-color flow cytometric analysis was performed in an FACSaria (BD Biosciences). The number of CD4+ T cells that had divided in response to IA2 805–817 or PBS (CFSE−) over 7 d that was IFN-γ when restimulated for 24 h by IA2 805–817 or RV-VP7 40–52 was enumerated and corrected to 20,000 CD4+ undivided (CFSE−) cells.

T cell cloning and peptide response assay

CD4+ T cell clones to RV-VP7 40–52 peptide were generated from PBMCs of an asymptomatic, 18-year-old at-risk male with Abs to GAD65 and IA2 and the highest-risk HLA genotype (A2,25; B44,39; DRB1*0301,0401; DQ2,8), as previously described (22). PBMCs at 105/ml in PBS were labeled with CFSE as above and placed into 96-well plates at 2 × 105/well in complete IMDM. RV-VP7 40–52 peptide was added to a final concentration of 10 mg/ml, the cells cultured for 10 d in 5% CO2 in air at 37°C, then pooled and labeled with anti-CD4 or isotype control mAb. CD4+ CFSE− cells (i.e., those that had proliferated in response to the peptide) were sorted by flow cytometry at 1 cell/well into 96-well plates precoated with 2 × 102 irradiated (50 Gy) JY feeder cells, 20 U/ml IL-2, 5 ng/ml IL-4, and 2.5 mg/ml PHA in complete IMDM. After 14 d in 5% CO2 in air at 37°C, growing cells were expanded every 3 to 4 d with IL-2 and IL-4.
as above, without PHA. Three of 15 clones that expanded the most were tested and confirmed for monoclonality by RT-PCR of the TCR clonotype as previously described (23). Cloned cells were taken 4 d after addition of cytokines, washed in PBS then IMDM, and resuspended in complete IMDM. Cells were added in triplicate at $2 \times 10^4$ cells/well to a 96-well U-bottomed plate, each well containing $2 \times 10^5$ PBMCs from an HLA-DR-DQ–matched donor as APCs and RV-VP7 40–52 or IA2 805–817 peptides (final 2 mg/ml). After incubation for 2 d in 5% CO$_2$ in air at 37˚C, cells were pulsed with $[^{3}H]$thymidine as above, harvested 16 h later, and counted.

**Statistical analysis**

The medians of subject groups were compared overall with the Kruskal-Wallis test and then for pairs of groups with Dunn’s posttest. Fisher’s exact tests were used to compare the frequencies of T cell responses, and a Bonferroni correction was made for multiple comparisons. Correlation was determined by Spearman rank-log test. Statistics were performed with GraphPad Prism software (version 3 for Macintosh, GraphPad, San Diego, CA).

**Results**

**Islet autoantigen and similar RV peptides bind to HLA-DR molecules associated with type 1 diabetes**

IA2 805–817 and GAD65 115–127, and the similar RV-VP7 peptides (Fig. 1), were bound to HLA class II molecules encoded by alleles DRB1*0401, DRB1*0404, and DRB4*0101 (also present on DRB1*04 haplotypes) for type 1 diabetes susceptibility, and to the protective allele DRB1*1501, but not to the susceptibility allele DRB1*0301 (Fig. 2, Table I). IA2 805–817 and RV-VP7 40–52 peptides had similar affinities for DRB1*0401 (weak), DRB4*0101 (moderate), and DRB1*0404 (strong and very strong). GAD65 115–127 and RV-VP7 16–28 peptides had similar affinities for DRB1*0401 (weak), DRB4*0101 (moderate), and DRB1*0404 (strong and very strong), with strong and moderate affinities, respectively, for DRB4*0101. IA2 805–817, GAD65 115–127, and RV-VP7 16–28 each bound strongly to the protective allele DRB1*1501, but RV-VP7 40–52 did not bind. Thus, overall, the RV-VP7 peptides had similar or stronger affinities for binding to HLA-DR4 susceptibility alleles than the respective sequence-similar IA2 and GAD65 epitopes.

**RV and autoantigen-derived peptides elicit similar frequencies of T cell proliferative responses**

T cell proliferative responses to IA2 805–817 and GAD65 115–127, and to the similar peptides in RV-VP7, are shown and summarized in Fig. 3. A substantial proportion of both islet autoimmune and control subjects, whose distribution of HLA-DR phenotypes is shown (Table II), responded to each peptide. Overall, the proportions of subjects responding or the mean responses did not differ between the groups, with the exception of a higher proportion of islet autoimmune responders to RV-VP7 40–52 (84% versus 60%; $p < 0.025$, Fisher’s exact test).

![FIGURE 2. Binding of sequence-similar peptides from IA2, GAD65, and RV-VP7 to HLA-DRB1*0401 (A), *0404 (B), *0301 (C), DRB4*0101 (D), and DRB1*1501 (E). Vertical dotted lines define the IC$_{50}$. Data are mean ± SEM; $n = 3$.](http://www.jimmunol.org/)

**Table I. Binding of IA2, GAD65, and RV-VP7 peptides to HLA-DR alleles**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>DRB1*0401</th>
<th>DRB1*0404</th>
<th>DRB1*0301</th>
<th>DRB4*0101</th>
<th>DRB1*1501</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA2 805–817</td>
<td>2.04</td>
<td>0.020</td>
<td>&gt;10</td>
<td>0.25</td>
<td>0.041</td>
</tr>
<tr>
<td>RV-VP7 40–52</td>
<td>1.07</td>
<td>0.0085</td>
<td>&gt;10</td>
<td>0.25</td>
<td>&gt;10</td>
</tr>
<tr>
<td>GAD65 115–127</td>
<td>0.18</td>
<td>0.022</td>
<td>&gt;10</td>
<td>0.018</td>
<td>0.012</td>
</tr>
<tr>
<td>RV-VP7 16–28</td>
<td>0.28</td>
<td>0.010</td>
<td>&gt;10</td>
<td>0.72</td>
<td>0.022</td>
</tr>
<tr>
<td>RV-VP7 12–24</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>8.2</td>
<td>&gt;10</td>
</tr>
<tr>
<td>RV-VP7 22–36</td>
<td>&gt;10</td>
<td>0.28</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>RV-VP7 28–40</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>4.2</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Indicator peptide

| 0.020 |

$[^{3}H]$Binding is expressed as IC$_{50}$ value, the concentration of peptide (µM) that inhibited binding of the indicator peptide (GAD65 555–567 or MBP 84–102 for DRB1*1501) by 50%.
exact test). There were no other differences between the two groups after stratification by HLA genotypes with Bonferroni correction for numbers of comparisons. T cell proliferative responses to IA2 805–817 and RV-VP7 40–52 correlated significantly (r = 0.63; p < 0.0001) as did responses to GAD65 115–127 and RV-VP7 16–28 (r = 0.38; p = 0.0008) (Fig. 4), but responses to IA2 805–817 and tetanus (r = 0.22; p = 0.06) and to GAD65 115–127 and tetanus (r = 0.17; p = 0.18) were not significantly correlated. These findings demonstrate that RV-VP7 sequences with similarity to IA2 and GAD65 auto-epitopes are also T cell epitopes and that T cell responses to them are frequent and similar in people with islet autoimmunity and in healthy individuals carrying HLA risk alleles for type 1 diabetes.

**T cells stimulated by the IA-2 epitope express IFN-γ when restimulated by the similar RV-VP7 peptide**

PBMCs were cultured for 7 d with or without IA2 805–817, or an irrelevant peptide, and then restimulated for 24 h with the same IA2 peptide, the similar RV-VP7 40–52 peptide, or no peptide. After restimulation with IA2 805–817, 271 CD4+ T cells that had divided (CFSEdim) per 20,000 undivided (CFSEbright) expressed IFN-γ (Fig. 5A). A similar number of CD4+ T cells, 212, expressed IFN-γ after restimulation with RV-VP7 40–52 (Fig. 5B). In contrast, the equivalent numbers of IFN-γ–positive CD4+ T cells after incubation initially with an irrelevant peptide (scrambled GAD65 115–127) were 8 and 7 (Fig. 5C, 5D) and without restimulation 17 and 6 (Fig. 5E, 5F), respectively. Thus, CD4+ T cells stimulated to divide by IA2 805–817 could be restimulated to express IFN-γ to the same extent by either IA2 805–817 or RV-VP7 40–52.

**RV-VP7 and IA2 epitopes are recognized by the same TCR**

Eleven clones were generated to the RV-VP7 40–52 epitope and tested for cross-reactivity with the IA2 805–817 epitope. Of these, two, JC1B2 and JC1B5, expressing a single Vβ 13 TCR, were restimulated to divide to the same or greater extent by IA2 805–817 (Fig. 6A, 6B). Proliferation of clone JC2B3 to RV-VP7 40–52 was inhibited 93% and 42%, respectively, by mAbs to HLA-DR and -DQ (Fig. 6C), consistent with HLA-DR restriction of the response.

**Discussion**

Molecular mimicry between nonself and self at the level of primary amino acid sequence similarities in viral and self proteins to elicit cross-reactive T cell responses has been hypothesized as a mechanism for breaking immune tolerance leading to autoimmune disease (24). In type 1 diabetes, enteric viruses such as Coxsackie B viruses (CBVs) and RVs have been proposed as potential etiological agents (13, 25–28). The P2-C protein of CBVs shares seven of nine aas at aa35–43 (KILPEVKEK) with GAD65 258–266 (KMFPEVKEK) (27), but a peptide from P2-C (aa33–52) that includes this motif bound very weakly to type 1 diabetes susceptibility HLA molecules; binding of the GAD65 sequence was not tested (29). In the autoimmune diabetes-prone NOD mouse (30), T cell cross-reactivity between the P2-C and GAD65 sequences was found, but CBV infection of NOD mice had no effect on T cell reactivity to the GAD65 peptide or on diabetes incidence (27). In humans with type 1 diabetes, CD4+ T cell clones generated to GAD65 258–266 showed no proliferation to CBV P2-C 35–43 (KILPEVKEK) with GAD65 258–266 (KMPEVKEK) (27), but a peptide from P2-C (aa33–52) that includes this motif bound very weakly to type 1 diabetes susceptibility HLA molecules; binding of the GAD65 sequence was not tested (29). In the autoimmune diabetes-prone NOD mouse (30), T cell cross-reactivity between the P2-C and GAD65 sequences was found, but CBV infection of NOD mice had no effect on T cell reactivity to the GAD65 peptide or on diabetes incidence (27).

### Table II. HLA class II phenotypes of subjects

<table>
<thead>
<tr>
<th>HLA-</th>
<th>Islet Autoimmune At Risk</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>DR3−4</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>DR4−4</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>DR3−3</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>DR4−X</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>DR3−X</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>DRX−X</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Not tested</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

*X* is not DR3 or −4.
have also been investigated for evidence of mimicry in type 1 diabetes. CD8+ T cell clones to GAD65 peptides, generated from subjects with type 1 diabetes, were cytotoxic in response to rubella virus peptides with weak sequence similarities to the GAD65 peptides (35). CD4+ T cell clones to multiple GAD65 peptides, generated from a patient with stiff-person syndrome, proliferated to a naturally processed, DR3-binding peptide from CMV (36), but the relevance of this finding to type 1 diabetes is unclear. Thus, the evidence for molecular mimicry as a potential mechanism of islet autoimmunity in type 1 diabetes is negative or at best circumstantial.

We suggest that the minimum criteria for mimicry at the T cell level are that a peptide from a candidate environmental agent

FIGURE 5. CD4+ T cells stained for intracellular IFN-γ following 7 d incubation of PBMCs from an islet autoimmune subject with IA2 805–817 peptide followed by IA2 805–817 for 24 h (A), IA2 805–817 peptide followed by RV-VP7 40–52 for 24 h (B), irrelevant peptide followed by IA2 805–817 for 24 h (C), irrelevant peptide followed by RV-VP7 40–52 for 24 h (D), IA2 805–817 peptide followed by no peptide for 24 h (E), and RV-VP7 40–52 peptide followed by no peptide for 24 h (F). The irrelevant peptide was a scrambled version of GAD65 115–127.

FIGURE 6. Proliferation of clones JC1B5 (A) and JC1B2 (B) to IA2 805–817 and RV-VP7 40–52 peptides and of clone JC2B3 to IA2 805–817 in the presence of mAb to HLA-DR or -DQ (C). Data are mean ± SEM; n = 3.
should elicit T cell responses in the context of a disease susceptibility HLA molecule similarly to the autoepitope peptide and that one TCR recognizes both peptides. We found that peptides in RV-VP7 with sequence similarity to epitopes in the islet Ags IA2 and GAD65 bound to HLA-DR4 molecules that confer susceptibility to type 1 diabetes and elicited proliferation of T cells from islet-autoimmune and healthy individuals expressing these HLA molecules. Parenthetically, this is the first report identifying RV T cell epitopes in humans. To address the more stringent criterion for mimicry, namely that one TCR recognizes both the self- and nonself peptide, we first showed that T cells from PBMCs that proliferated to IA2 805–817 could be restimulated to express IFN-γ by both IA2 805–817 and RV-VP7 40–52. That recognition could occur via the one TCR was confirmed by showing that two T cell clones generated to RV-VP7 40–52 also proliferated in response to IA2 805–817 presented in the context of DRB1*0401. These findings meet the minimum criteria for molecular mimicry.

Stratification of responses by HLA revealed that both RV-VP7 peptides were T cell epitopes not only in DRB1*0401 individuals, but also in some with DRB1*0301 without DRB1*0401/4, despite the lack of binding of peptides to DRB1*0301. This suggests that peptide presentation could also occur by HLA alleles at other loci on the same genotype, such as HLA-DRB3, DQ2, or -DP. HLA-DRB1*1501 is protective in type 1 diabetes, but the mechanism of this effect remains unexplained. Interestingly, both autoantigen peptides and RV-VP7 16–28 bound strongly to DRB1*1501, but RV-VP7 40–52, similar to IA2 805–817, did not bind. Therefore, if mimicry between RV-VP7 40–52 and IA2 805–817 is involved in promoting islet autoimmunity, it may be less likely to occur in DRB1*1501 individuals. To examine this possibility, T cell responses to RV-VP7 40–52 and IA2 805–817 could be measured in subjects who are DRB1*1501, but not DRB1*0401. In the current study, this was precluded by HLA matching.

A role for molecular mimicry implies a higher frequency and/or increased magnitude of T cell responses to the environmental epitope in islet-autoimmune subjects than controls; however, this was the case only for RV VP7 40–52. On the other hand, the relatively high frequency of T cell responses in controls and islet-autoimmune subjects may not be unexpected given that the controls were HLA similar. Together with lack of correlation between T cell responses to peptides and tetanus toxoid, this indicates that the islet-autoimmune subjects did not have a general increase in T cell reactivity. Future studies to determine the functional properties of T cells that respond to RV-VP7 and autoepitope peptides might reveal differences between islet-autoimmune and control subjects. There is no evidence for more frequent or more persistent RV infection in islet-autoimmune subjects, which may be a contributing factor but not sufficient condition. Martinuzzi et al. (19) reported that IA2 805–813 was a subdominant HLA-A2–restricted CD8+ T cell epitope in patients with recent-onset type 1 diabetes, becoming the dominant epitope after several months, and that GAD65 114–123 was the dominant HLA-A2–restricted epitope at onset but was subdominant at follow-up (19). These findings strengthen the case for mimicry between the RV-VP7 and IA2 and GAD65 peptides. We suggest that these IA2 and GAD65 peptides could be combiotopes for both CD4+ and CD8+ T cells restricted by HLA class II and I molecules, respectively. Although direct, unequivocal proof for molecular mimicry in human autoimmune disease is probably unattainable, our findings support the hypothesis that mimicry with RV may contribute to the pathogenesis of type 1 diabetes.