ASSOCIATION BETWEEN HLA-CLASS II ALLELES AND T-CELL PROLIFERATION IN RESPONSE TO ENTEROVIRUSES AND ADENOVIRUS ANTIGENS IN NEWLY DIAGNOSED CHILDREN WITH TYPE 1 DIABETES MELLITUS

Eman M. Saleh1    Nidhal A. AL-Mohymen2

1Department of Microbiology, AL-Kindy College of Medicine, Baghdad University
2Department of Microbiology, College of Medicine, AL- Nahrain University

ABSTRACT
Viruses may be involved in the pathogenesis of Type 1 Diabetes Mellitus (T1DM), either through direct β-cell infection or as triggers of autoimmunity. T-cell proliferation was evaluated in response to Enteroviruses antigens including Coxsackievirus B and Poliovirus in addition to Adenovirus in an HLA-matched population of children with T1DM and in children who were apparently healthy. A total of 60 Iraqi T1DM children were included in the present study. They were with new onset of the disease. For the purpose of comparisons, 50 apparently healthy control subjects were also selected. HLA typing was measured by microlymphocytotoxicity, while methylthiazoltetrazolium (MTT) assay was used for lymphocyte proliferation by culturing peripheral blood lymphocytes (PBL) with Coxsackievirus B5, Adenovirus 3, 4 and 7 and Poliovaccine. No significant differences were shown in the PBL proliferative percentage in response to Con-A mitogen and tested viruses (CVB5 and Adenovirus) between T1DM and healthy controls, but PBL proliferative percentage of patients showed a significant decline in response to Poliovaccine. HLA class II (-DR3, DR4, DQ2 and DQ3) antigens were significantly increased in T1DM patients and they played an important role in the etiology of the disease. Strong T-cell proliferation in response to the tested viral antigens were observed to be related to HLA-DR4 and HLA-DQ3 antigens, whereas the HLA-DR3 and HLA-DQ2 alleles were associated with weak responsiveness to the same antigens. However, in children with new-onset diabetes, responses were decreased and this could be caused by trapping of virus-specific T-cells in the pancreas.

Key words: T1DM, HLA, Lymphocyte proliferation, Enteroviruses, Adenovirus.
العلاقة بين مستضدات التطابق النسيجي- الصنف الثاني والفعالية الوظيفية للخلايا اللمفية بعد تحقیقها بمستضدات حمایة ومعایة في الاطفال المصابین حديثاً بالسكري من النوع الأول

Eman M. Saleh  and  Nidhal A. AL-Mohyneen

الخلاصة

أثبتت العديد من الدراسات أن الفيروسات دوراً تؤديه في حدث امراضية مرض السكر من النوع الأول بالاصابة المباشرة لخلايا بيتا في البنكرياس أو بتحفيز المناعه الذاتیة. أجريت الدراسة لغرض تقييم الفعالية الوظيفية للخلايا اللمفية بعد تحقیقها بمستضدات الجمّات المعوية والتي تشمل فيروس الكوكساكي نوع-B وفیروسات شلل الأطفال فضلا عن الحمایة الذاتیة في مجموعة من الأطفال المصابین بمرض السكر من النوع الأول وجموعة من الأطفال للاصحاء المصابين بمرض السكر النوع الأول فضلا عن مجموعة السيطرة المكونة من 50 طفلاً بدون أصحة ظاهرية لغرض التحقيق

تم قياس الفعالية الوظيفية لمخلايا الممفية بعد حضنها مع الفيروسات المنفة الذكر . ظهرت النتائج انخفاضاً غير معنیاً في الفعالية الوظيفية للمخلايا اللمفیة بعد حضنها مع الفيروسات Анفة الذكر . أظهرت النتائج انخفاضاً غير معنیاً في الفعالية الوظيفیة للمخلايا اللمفیة كاستجابة للمشط، وكذلك بعد حضنها مع فيروس الكوكساكي النوع المصلي ب-5 وفیروسات الحمایة الذاتیة للاتباع المیلية 3، 4، 5 في الأطفال المصابین بالسكري . ولكن هذا الانخفاض كان معنیاً فقط عند استخدام فيروسات شلل الأطفال. هناك زيادة معنیاً في نسبة الأطفال الحامین لالیمات الخطرة من الصنف الثاني مقارنة بالصحیة.
INTRODUCTION

There are a considerable body of evidences suggesting that involvement of several groups of viruses including: Congenital rubella, Rotaviruses, Retroviruses, Herpes viruses, Cytomegalovirus, Measles, Hepatitis C, and particularly those of the Enterovirus genus, in the development and / or acceleration of Type 1 Diabetes Mellitus (T1DM) (1). Several epidemiological and prospective studies showed that some cases of T1DM are strongly associated with Enterovirus (EV) infections (2), and the children who later developed T1DM had more EV infections than control children years before the diagnosis of the disease (3). Coxsackie virus B4 (CVB4)- specific IgM responses are more common in newly diagnosed subjects with T1DM than in healthy control subjects (4). The finding of viral RNA in circulation at the onset of the disease have further support the role of Enteroviruses (5). Several mechanisms have been proposed to explain this putative link with T1DM pathogenesis, including molecular mimicry (6), bystander activation through release of autoimmune mediators like proinflammatory Cytokines IL-1β; TNF-α; and IFN-γ (7), and super antigen effect (8).

Viral infection like other environmental risk factors, can probably induce β-cell damaging processes only in individuals with genetic T1DM susceptibility. The most important risk genes locate within the HLA gene complex, where HLA-DQ alleles associated with increased susceptibility to or protection against T1DM can be defined (9). Enterovirus infections possibly occur predominantly in individuals with the DQA1*0501. DQB1*02 haplotype, who usually are also positive for HLA-B8 and HLA-DR3 alleles (10,11). HLA may also influence immune responses to EV antigens in comparison occurs between patients and control individuals (12).

Few studies had focused on T- cell / virus interaction. In the present study T- cell proliferation in response to Enterovirus antigens including Coxsackie virus B and Poliovirus in addition to Adenovirus was analyzed in an HLA- matched population of children with T1DM and children who were healthy.

MATERIALS AND METHODS

Sixty Iraqi T1DM children were subjected to this study. The patients were attending to National Diabetes Center at Al-Mustansiriya University during the period May 2004 to October 2005. Their ages ranged from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). For the diagnosis of Diabetes Mellitus, the criteria as listed in the (13), was used. All the patients were treated with daily replacement doses of Insulin at the time of blood sampling. For the purpose of comparisons, 50 apparently healthy control subjects matched for age (4-17) years, sex and ethnic back ground (Iraqi Arabs) were selected who had no history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group. The patients and control subjects were divided into two groups according to their ages, equal or less than 10 years and more than 10 years old.
Collection of blood samples

Eight milliliters of blood was placed in heparinised test tube (10 U/ml) used for lymphocyte separation for the detection of HLA polymorphism and lymphocyte proliferation. Heparinised blood was processed as soon as possible.

HLA typing: were measured by microlymphocytotoxicity assay as described by Stoker and Bernoco (14).

Lymphocyte proliferation using methylthiazoltetrazolium (MTT) assay

Heparinized venous blood was collected, and PBLs were isolated using Ficoll- isopaque gradient centrifugation (Flow-Laboratories ,UK). The washed PBLs were resuspended in complete RPMI- 1640 medium (Euroclone, UK) supplemented with 10% AB serum (National blood transfusion center); Hepes; Crystalline penicillin (1,000,000 IU) and Streptomycin (1gm)(Pharma-intersprl, Belgica), the final lymphocyte concentration was adjusted to 1-2x10^6 cells / ml.

Triplicate incubations of 1-2x10^6 PBL/ ml with antigen(s) in a final volume of 100μl continued to incubate 96 flat-bottom microculture plates for 3 days at 37°C in a humidified 5% CO₂ incubator. Then 20μl of (MTT) 1-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (Sigma, Germany) working solution was added to each culture well and the culture were incubated for further 4 hrs. The converted dye was solubilized by adding acidic isopropanol. The absorbancy was read using microculture plate reader using a wave length of 570 nm (15).

Antigens

- Coxsackievirus B5 (CVB5) antigen solution (1:5 dilution), (KBR-CF antigen Vero, France).
- Poliovirus Trivalent Vaccine (1:5 dilution), (Polioral Trivalent; Chiron).
- Adenovirus type 3,4,7 solution (1:10 dilution), (KBR-CF antigen type 3, 4, 7, Vero).
- Concavalin-A (Con-A) mitogen (100 μg/ml), was used as a mitogen positive control.

: The final concentration or dilution for the three viral antigens was achieved according to the result of MTT serial dilution run of these antigens.

The Note percent of proliferative response of lymphocytes was calculated by the following formula (15):

\[
\% \text{ Proliferation} = \left( \frac{\text{Absorbancy of experimental wells}}{\text{Absorbancy of control wells}} - 1 \right) \times 100
\]

Statistical analysis

Regarding HLA and disease association the frequency distribution for selected variables was done. Student t-test was used to measure the differences between two means, the results were expressed as means ± standard error (SE). The single factor ANOVA (F-test) was used in this study to find out whether the difference among more than two groups of samples is significant or not, and Pearson Correlation (R), which measures to what degree the two variable observations are correlated to each other, and the type of this correlation.
RESULTS

Lymphocyte Proliferation

This test was performed to study whether the different viral antigens have any association with proposed cell mediated immune (CMI) activation or not after incubation with peripheral blood lymphocytes (PBLs) of T1DM patients and healthy controls. The results of mean proliferative percentage in response to Con-A were represented in Table (1). A similar mean lymphocyte proliferation percentage in response to Con-A mitogen was seen among patients and control groups, but newly diagnosed T1DM patients tended to have a lower non significant proliferative percentage than control subjects ≤10 years old (83.33 vs. 85.93% respectively, P₁=0.82) and in >10 years old group (86.04 vs. 92.7% respectively, P₁=0.62).

Table (1) : t-test between controls and T1DM patient groups regarding comparison of MTT proliferation percentage in response to Con-A.

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Age ≤10 years</th>
<th>Age &gt;10 years</th>
<th>P₁</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>Con-A</td>
<td>Controls</td>
<td>21</td>
<td>85.93</td>
<td>10.60</td>
</tr>
<tr>
<td></td>
<td>T1DM</td>
<td>36</td>
<td>83.33</td>
<td>5.60</td>
</tr>
</tbody>
</table>

P₁: T1DM patients vs. control
P₂: T1DM patients ≤10 years vs. patients >10 years old.

Role of Viral Antigens in Functional Activation of PBL

Considering the response to different viral antigen, a lower mean proliferative percentage was seen among patients ≤10 years old in response to CVB₅ compared to controls (36.67 vs. 49.16% respectively) and among patients >10 years old than controls (38.87 vs. 51.20% respectively). This differences failed to reach significant levels in both age groups (P₁=0.061, P₁=0.14 respectively) ,Table (2).

Significant decline of proliferative response against Poliovaccine was seen in T1DM patients (34.44%) than controls (47.38%) (P₁= 0.045) in ≤10 years old group and (28.30 vs. 40.86% respectively, P₁= 0.004 in >10 years old group ,Table (2).
Table (2) : Comparison of mean proliferation percentage of PBL between controls and T1DM patients in response to CVB5, poliovaccine and adenovirus.

<table>
<thead>
<tr>
<th>Viral Antigens</th>
<th>Age ≤10 years</th>
<th>Age &gt;10 years</th>
<th>P&lt;sub&gt;1&lt;/sub&gt;</th>
<th>P&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVB5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>49.16</td>
<td>5.88</td>
<td>0.06</td>
</tr>
<tr>
<td>T1DM</td>
<td>36</td>
<td>36.67</td>
<td>3.08</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polio Vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>47.38</td>
<td>5.83</td>
<td>0.04</td>
</tr>
<tr>
<td>T1DM</td>
<td>36</td>
<td>34.44</td>
<td>2.79</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeno-virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>20.67</td>
<td>2.24</td>
<td>0.82</td>
</tr>
<tr>
<td>T1DM</td>
<td>36</td>
<td>19.97</td>
<td>1.61</td>
<td>0.82</td>
</tr>
</tbody>
</table>

P<sub>1</sub>: T1DM patients vs. control
P<sub>2</sub>: T1DM patients ≤10 years vs. patients >10 years old.

A non significant proliferative percentage decline in response to adenovirus was observed in patients (19.97%) and controls (20.67%) (P<sub>1</sub> = 0.82) in ≤10 years old group and also in patients >10 years old (23.02%) in comparison with controls (28.61%) (P<sub>1</sub> =0.23).

No statistical differences appeared in the mean lymphocyte proliferative percentage between patients in both age groups against CVB5 (P<sub>2</sub> =0.57), Poliovaccine (P<sub>2</sub> =0.14) and Adenovirus (P<sub>2</sub> =0.35).

**Mitogenic Properties of Tested Viral Antigens In Vitro**

To confirm the immunostimulatory effect of CVB5, Polio and Adenovirus, which compared with Con-A mitogen as a control positive for PBL mitogenesis and with control negative (Table-3). Statistical analysis, had shown that CVB5 had a mitogenic potential *in vitro*. By comparing the mean of MTT OD value of CVB5 (0.369) with control negative (C-ve) (0.270) in patient group ≤10 years old, it was found that control negative is significantly lower than CVB5 mean of MTT reading (P<sub>1</sub> = 0.045). The same statistical difference was seen among patients >10 years old between OD value of CVB5 and control negative (0.368 vs. 0.265, P<sub>2</sub> =0.037). This indicates that CVB5 may have a role in inducing the disease in those patients. The mean MTT reading of CVB5 was significantly lower than Con-A mean MTT reading in patients ≤10 years old (0.369 vs. 0.495 respectively, P<sub>1</sub> =0.045) and (0.368 vs. 0.493 respectively, P<sub>1</sub> =0.045) in patients >10 years old. This means that CVB5 has a good mitogenic potential, but does not reach the high level of Con-A.
Concerning the Poliovaccine and Adenovirus, it was found that control negative mean of MTT OD value (0.270) was lower than Poliovaccine (0.369) and Adenovirus (0.324) mean of MTT readings in patients group ≤10 years old. These means were weakly significant among Poliovaccine (P₁ = 0.054) and not statistically different among Adenovirus. On the other hand, the same results were demonstrated among patients >10 years old, (0.265 vs. 0.340, P₂ = 0.074) for Poliovaccine and (0.265 vs. 0.326 P₂ = 0.17) for Adenovirus. This indicates that compared with that of CVB₅, although Poliovaccine had a weak mitogenic potential in patients group ≤10 years old and might have weakly immunostimulatory activity in vivo, Table(3).

Lymphocyte proliferation percent against both Poliovaccine and Adenovirus showed significant positive correlation with lymphocyte proliferative percent in response to CVB₅ (r =0.38, r = 0.25 respectively, P<0.05), nevertheless a positive correlation between Poliovaccine and Adenovirus (r = 0.45, P<0.05).

Relation of HLA class II alleles with the PBL proliferation percentage in T1DM patients:

At HLA-class II region (DR-loci), highly significant increased frequencies of DR3 (53.33 vs. 26.25%) and of DR4 (50.0 vs. 12.5%) were observed in the patients (P=9.7x10⁻³ and 1x10⁻⁻ respectively) (data was not shown). At HLA-DQ loci, two antigens DQ2 and DQ3 were significantly increased in the patients compared with controls (33.33 vs. 15.0%, P=0.009) for DQ2 while (40.0 vs. 20.0%, P=0.008) for DQ3. The distribution of HLA-DR and -DQ antigens in T1DM children and controls were represented in Tables (4 , 5).

Table (4) : Distribution of HLA-DR antigens in T1DM children and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>DR3/DR4</th>
<th>DR3</th>
<th>DR4</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1DM (60)</td>
<td>25</td>
<td>7</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Controls (50)</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>
Table (5) : Distribution of HLA-DQ antigens in T1DM children and control groups.

<table>
<thead>
<tr>
<th>groups</th>
<th>DQ2/DQ3</th>
<th>DQ3</th>
<th>DQ2</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1DM (60)</td>
<td>9</td>
<td>15</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>Controls (50)</td>
<td>-</td>
<td>12</td>
<td>9</td>
<td>29</td>
</tr>
</tbody>
</table>

To find out any relation between the HLA-class II risky alleles (genetic factors) and proliferative percentage of MTT (CMI level), ANOVA test was applied to compare the proliferative percentage in patients with HLA-DR risky alleles (DR3; DR4 and DR3/DR4) with those patients who had other alleles. Results represented in Table (6) showed that the mean PBL proliferative percentage in response to different tested viral antigens were significantly higher in the patients with DR4, DR3 and DR3/DR4 serotypes compared with the children carrying other alleles. The significant levels scored (P= 0.021) in response to CVB5, (P=0.031) in response to Poliovaccine, and (P= 0.041) in response to Adenovirus. Moreover, the mean proliferative percentage were significantly higher in patients carrying DR4 allele than those in patients with DR3 alleles in response to CVB5 (62.67 vs. 43.32%, P=0.038), to Poliovaccine (59.86 vs. 38.40%, P=0.031) and to Adenovirus (46.02 vs. 22.48%, P= 0.046).

Table (6) : Relation of mean lymphocyte proliferation percentage in response to different viral antigens with the HLA-DR risky alleles in T1DM patients.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>DR3/DR4 (n=25)</th>
<th>DR3 (n=7)</th>
<th>DR4 (n=5)</th>
<th>Others (n=23)</th>
<th>ANOVA F-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVB5</td>
<td>40.37</td>
<td>43.32</td>
<td>62.27</td>
<td>29.73</td>
<td>8.585</td>
<td>0.021 (S)</td>
</tr>
<tr>
<td>Polio vaccine</td>
<td>34.42</td>
<td>38.4</td>
<td>59.86</td>
<td>25.27</td>
<td>7.689</td>
<td>0.033 (S)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>29.44</td>
<td>22.48</td>
<td>46.02</td>
<td>26.14</td>
<td>5.704</td>
<td>0.041 (S)</td>
</tr>
</tbody>
</table>

Concerning the HLA-DQ risky alleles (DQ2, DQ3,and DQ2/DQ3), our results represented in table (7) showed a significant increase of proliferative percentage in patients carrying different HLA-DQ risky alleles compared with the patients who lack these alleles. The results scored as significant levels of (P= 0.032) in response to CVB5, (P= 0.038) in response to Poliovaccine, and (P= 0.042) in response to Adenovirus.

Table (7) : Relation of mean lymphocyte proliferation percentage in response to different viral antigens with the HLA-DQ risky alleles in T1DM patients.

<table>
<thead>
<tr>
<th>Viruses</th>
<th>DQ2/DQ3 (n=9)</th>
<th>DQ3 (n=15)</th>
<th>DQ2 (n=11)</th>
<th>Others (n=25)</th>
<th>ANOVA F-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVB5</td>
<td>42.84</td>
<td>60.90</td>
<td>26.41</td>
<td>33.63</td>
<td>7.975</td>
<td>0.032 (S)</td>
</tr>
<tr>
<td>Polio vaccine</td>
<td>39.31</td>
<td>48.09</td>
<td>23.21</td>
<td>27.29</td>
<td>6.695</td>
<td>0.038 (S)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>22.26</td>
<td>37.41</td>
<td>31.37</td>
<td>26.74</td>
<td>5.684</td>
<td>0.042 (S)</td>
</tr>
</tbody>
</table>
As detected in table (7), the proliferative percentages were significantly higher in patients with DQ3 alleles than in patients with DQ2 alleles in response to all tested viral antigens.

**DISCUSSION**

**Functional Activity of PBL**

The use of lymphocyte proliferation is one of the more frequently used "in vitro" techniques for the study of the specific and non-specific stimulation capability of lymphocytes(15). The technique is based on the capability of the lymphocytes for responding to an antigen (specific response) which has induced memory lymphocyte, either by vaccination or by natural infection. These lymphocytes, when they are repeatedly contacted with antigens, have a blastogenic transformation (16).

MTT has been used in the measurement of proliferative percentage of PBL which has been found lower in T1DM patients than in healthy controls in response to Con-A. Considering the responses to viral antigens, proliferative responses against CVB₅ and adenovirus were tended to have a lower percentage in T1DM patients than controls, but these values were not statistically different, while the proliferative responses against Poliovaccine was significantly lower in patients especially in >10 years old group than controls. The low proliferative responses against CVB₅ antigen at disease onset is in agreement with other studies showing reduced T-cell proliferation against CVB₄ (17), while the same investigators found in previous study, no differences in T-cell proliferation against CVB₄-infected lysate between diabetic patients and healthy-non diabetic individuals (6). Another report conducted by Juhela, et al. (18) found that PBL of the children at onset of T1DM had significant weaker responses to purified CVB₄ and non-significant decrease in response to Poliovirus type 1 and 3 than healthy children, while the responses to Adenovirus did not differ between patients and controls. Temporary decline in T-cell responsiveness at diabetes onset has also described in glutamic acid decarboxylase (GAD) peptide that contains the homology region to the CVB₄ 2C protein (19).

The present study results are open to several interpretations. One explanation is that, decreased responses of PBL are due to redistribution of virus-specific T-cells, with virus-responder cells presumed to have homed to the Pancreas and therefore unavailable for detection in peripheral blood (8), and so T-cell responses to various viral antigens may be suppressed at the onset of the disease. On the other hand, Varela-Calvino and his team(17) in their study indicated abundance of circulating primed CVB₄ specific responder T-cells that secretes IFN-γ in T1DM patients with relative lack of proliferation. These finding have been related to two broadly defined phenotypes of memory T-cells characterized by Sallusto and Lanzavecchia(20). The Primed (memory) T-cells with the capacity to proliferate termed as "central memory" TCM cells. These cells lack immediate effector function and predominantly produce IL-2, the major T-cell growth factor to support proliferation and express CCR₇, a chemokine receptor, that direct homing to lymph nodes. In contrast the primed memory cell subsets that produces the proinflammatory Cytokines IFN-γ during an immune response termed "effector memory" subset TEM, those cells do not express CCR₇, present in the circulation at sites of infection or tissue inflammation and release Cytokines.
On the other hand most studies showed that CVB5 infection caused meningitis, respiratory, gastrointestinal and cardiac diseases, while T1DM most commonly caused by CVB4 and CVB3 (21). Our result showed that CVB5 may be associated with the disease.

Relation of Lymphocyte Proliferation with HLA

The present results indicated that stronger T-cell proliferation in response to CVB5, Poliovaccine and Adenovirus were related to HLA-DR4 allele and HLA-DQ3 allele; whereas the HLA-DR3 and HLA-DQ2 were associated with weak responsiveness to the same antigens. These results are in agreement with the report of Bruserud et al.,(12) who found that DR4, which is in linkage disequilibrium with the HLA-DQB1*0302 allele, associates with strong T-cell responses; whereas HLA-DR3 associated with HLA-DQB1*02 allele associates with weak T-cell responses to Enterovirus antigens. Juhela and her team(18), also reported the same observation in T-cell responses to Enterovirus antigens in T1DM patients.

REFERENCES