Identification of HLA-A*01- and HLA-A*02-restricted CD8+ T-cell epitopes shared among group B enteroviruses

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Acute enteroviral infections ranging from meningitis, pancreatitis to myocarditis are common and normally well controlled by the host immune system comprising virus-specific CD8+ cytotoxic T lymphocytes (CTL). However, in some patients enteroviruses and especially coxsackieviruses of group B are capable of inducing severe chronic forms of diseases such as chronic myocarditis. Currently, it is not known whether divergences in the CTL-related immune response may contribute to the different outcome and course of enterovirus myocarditis. A pre-requisite for the study of CTL reactions in patients with acute and chronic myocarditis is the identification of CTL epitopes. In order to define dominant enterovirus CTL epitopes, we have screened, by using gamma interferon (IFN-γ) ELISPOT, 62 HLA-A*01- and 59 HLA-A*02-positive healthy blood donors for pre-existing CTL reactions against 12 HLA-A*01 and 20 HLA-A*02 predicted CTL epitopes derived from coxsackieviruses of group B. Positive CTL reactions were verified by FACS analysis in a combined major histocompatibility complex-tetramer IFN-γ staining. A total of 14.8% of all donors reacted against one of the three identified epitopes MLDGHLIAFDY, YGDDVIASY or GIIYIIYKL. The HLA-A*02-restricted epitope ILMNDQEVGV was recognized by 25% of all tested blood donors. For this peptide, we could demonstrate specific granzyme B secretion, a strong cytolytic potential and endogenous processing. All four epitopes were homologous in 36–92% of group B enteroviruses, providing a strong basis for monitoring the divergence of T-cell-based immune responses in enterovirus-induced acute and chronic diseases.

INTRODUCTION

Enteroviruses are non-enveloped, positive-strand RNA viruses that belong to the family Picornaviridae. Currently, the enterovirus genus represents 68 human serotypes classified into five species [A–D and polioviruses (Stanway et al., 2005)] differing by about 40% in their nucleotide sequence (Simmonds & Welch, 2006). Enteroviral evolution occurs through genetic drift (Chua et al., 2001), which is primarily prominent in group B enteroviruses (Simmonds & Welch, 2006), including coxsackieviruses of group B (CVB). In addition, the occurrence of interserotypic recombination events between different coxsackievirus strains has also been reported (Bouslama et al., 2007).

It has been estimated that 5–10 million symptomatic infections with non-polio enteroviruses occur annually in the USA (Strikas et al., 1986). Although most of these infections are associated with a mild febrile illness or upper respiratory infection (Rotbart et al., 1999), enteroviruses can also cause severe, potentially fatal diseases such as acute flaccid paralysis [caused by CVB in 13% of all cases (Saeed et al., 2007)], aseptic meningitis [caused by enteroviruses in up to 90% of all cases (Rotbart, 1995)] or acute viral myocarditis with CVB3 as one of the primary causative agents (Baboonian et al., 1997; Kim et al., 2001; McManus et al., 1991). Whereas in adults acute CVB myocarditis is rarely lethal, children are particularly susceptible to diverse CVB infections with mortality rates due to myocarditis in the range of 30–50% (Modlin & Rotbart, 1997). In most patients acute myocarditis is abrogated by the successful
elimination of the virus, but in some patients the viruses may persist and thus, the disease may progress to chronic myocarditis and finally to dilated cardiomyopathy (DCM) (incidence of six per 100,000 in North America) (Codd et al., 1989; Klingel et al., 2004), with DCM accounting for approximately 50% of all heart transplantations (Boucek et al., 2001).

Hitherto, nothing is known about a possible correlation between the outcome and course of enterovirus myocarditis and the efficacy of T-cell-mediated immune responses. This is mainly due to the lack of information about enteroviral cytotoxic T lymphocyte (CTL) epitopes being a pre-requisite for a profound monitoring of T-cell-based immune reactions.

The relevance of the CD8+ T cells for an efficient elimination of CVB3 during the acute phase of myocarditis was demonstrated in the mouse model of enterovirus myocarditis (Klingel et al., 2003). Moreover, the severity of CVB3 myocarditis was found to be magnified in CD8−/− mice (Opavsky et al., 1999), suggesting a dominant role of CD8+ T cells in the protection against enterovirus challenge. On the other hand, it has been suggested that an exuberant immunological reaction – e.g. massive tumour necrosis factor alpha secretion by T cells infiltrating the myocardium – can result in collateral damage of the myocardium, which is dominantly immuno-mediated (Knowlton & Badorff, 1999).

Up to now, only one human enteroviral CTL epitope is known: EVREKHEFL (P2C_1138–1146, CVB4) (Varela-Calvino et al., 2004) which, however, is only present in less than 25% of all group B enteroviruses. Due to this lack of known major histocompatibility complex (MHC) class I-restricted T-cell epitopes, mouse studies have been performed with recombinant CVB3 viruses expressing dominant lymphocytic choriomeningitis virus epitopes (Kemball et al., 2008; Slifka et al., 2001). Here, a CTL-mediated reduction of virus loads by up to 50-fold was observed (Slifka et al., 2001).

Knowledge of enteroviral CTL epitopes is a major prerequisite for molecular defined approaches, which aim at identifying immunological factors contributing to the protection of a chronic course of myocarditis. Thus, in order to provide a profound basis for studying the role of CTL in infections with CVB3 and other group B enteroviruses, we have screened healthy blood donors for the presence of dominant CVB3 CTL epitopes.

METHODS

Peptide selection and synthesis. CTL epitopes of CVB3 (SWISS-PROT accession POLG_CXBN) were predicted using the SYFPEITHI matrix (www.syfpeithi.de). The predictions were performed for HLA-A*01 nona-, deca- and undecamers as well as for HLA-A*02 nona- and decamers. All peptides with a SYFPEITHI score above 26 were synthesized as described previously (Schirle et al., 2000) and used for ELISPOT assays (Supplementary Table S1 available in JGV Online).

Isolation of peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from buffy coats or leukapheresis products by standard ficoll gradient separation and cryopreserved in fetal calf serum with 10% DMSO until use. Screening of PBMCs was approved by the local ethic’s committee.

Pre-sensitization of PBMCs. T-cell culture medium consisted of Iscove’s modified Dulbecco’s medium, 50 U penicillin ml−1, 50 μg streptomycin ml−1 and 20 μg gentamicin ml−1 (all from Lonza), 50 μM 2-mercaptoethanol and 10% heat-inactivated human AB serum (CC pro). PBMCs were pre-sensitized for 12 days with peptide prior to the actual ELISPOT experiment. Thawed PBMCs were taken into culture on day 1 with 5 ng interleukin (IL)-4 ml−1 and 5 ng IL-7 ml−1 (both Promokine), adapted from Coulie et al. (2001). On day 2 the same amount of cytokines together with 1 μg peptide ml−1 was added. IL-2 (2 ng μl−1) was added on days 4 and 6. Cells were used for ELISPOT on day 13.

ELISPOT assay. PBMCs were stimulated for 13 days with HLA-matched cytomegalovirus (CMV), Epstein–Barr virus (EBV) and influenza A virus peptides (CEF-positive mix), with a human immunodeficiency virus (HIV) peptide and the CVB3 peptides of interest. In the ELISPOT assay, 500’000 pre-sensitized cells were cultured for 24 h with 1 μg ml−1 per peptide and as a positive control 10 μg phytohaemagglutinin (PHA)-L ml−1 was used. IFN-γ was detected using the Human IFN-γ ELISPOT kit (MabTech) according to the manufacturer’s recommendation and the cancer immunotherapy monitoring panel (Britten et al., 2008). Reactions were regarded as positive if the mean number of spots per well was at least 10 and more than three times the mean number of spots of the negative control using an HIV peptide. Granzyme B release was detected using the Human Granzyme B ELISPOT kit (MabTech). ELISPOT experiments were performed using 500’000 autologous PBMCs, which were infected for 1 h with CVB3 at an m.o.i. of 10.

T2-binding assay. T2 cells were washed twice in serum-free medium. Per well, 2.5 × 105, T2 cells were incubated for 45 min with 1 μM fluorescein isothiocyanate (FITC)-labelled control peptide and peptide of interest (0.01–20 μM) at room temperature. Afterwards cells were washed with PBS and analysed directly on a FACSCanI (BD Biosciences).

FACS analysis and intracellular IFN-γ staining. Biotinylated recombinant HLA class I molecules and fluorescent HLA tetramers for CD8+ T-cell analysis were produced as described previously (Altman et al., 1996). Briefly, fluorescent tetramers were generated by co-incubating biotinylated HLA monomers with streptavidin–phycoerythrin (PE) or streptavidin–allophycocyanin (APC) (Molecular Probes) at a 4:1 molar ratio. Autologous PBMCs were pulsed with 10 μg peptide ml−1 and incubated in the presence of Golgi-Stop (BD Biosciences) for 6 h. Cells were analysed using a Cytofix/Cytoperm Plus kit (BD Biosciences) and CD8–PE-Cy7, CD4–FITC, IFN-γ–PE (all BD Biosciences) and HLA-tetramer–APC. For negative controls, cells were incubated with either HIV or CEF peptides, respectively. Stimulation with phorbol-12-myristate-13-acetate (PMA)/ionomycin was used as a positive control. Cells were analysed on an eight-colour FACSCantoII.

Vital assay. Vital assay was performed as described elsewhere (Hermans et al., 2004). In brief, PBMCs were pre-sensitized as described with ILMNDQEVGV, HIV and CEF peptides. Target cells were washed in serum-free medium and labelled with carboxyfluorescein diacetate succinimidyl ester (CFSE), Far Red or with both CFSE and Far Red according to the manufacturer’s instructions (Invitrogen). Cells were loaded with 10 μM peptide overnight. ILMNDQEVGV-loaded T2 cells (CFSE-labelled) were used as specific targets, HIV-loaded T2 cells (Far Red-labelled) served as a negative control.
control and CEF-loaded T2 cells (CFSE + Far Red-labelled) represented the positive control. Each peptide ($2 \times 10^5$)-loaded T2 cell group was incubated for 24 h with varying amounts of pre-sensitized PBMCs (300–300 000) and subsequently analysed by FACS analysis on a FACS Cantor II. The specific lysis was calculated according to Hermans et al. (2004).

RESULTS AND DISCUSSION

Initial screening procedure

In order to identify immunodominant T-cell epitopes from CVB3, the whole proteome of CVB3 was screened for HLA-A*01 and HLA-A*02 candidate epitopes using the SYFPEITHI routine. The SYFPEITHI routine expects the optimal T-cell epitope within the 10 high-scoring peptides of each protein. Naturally presented T-cell epitopes are assumed to appear among the top 2 % of peptides in the high score list in more than 90 % of predictions (Rammensee et al., 1999). Furthermore, a variant of the only known enteroviral cytotoxic T-cell epitope EVREKHEFL was included in our analysis (SYFPEITHI score HLA-A*02 = 14). This peptide bears a K/R exchange in position three that naturally occurs for example in CVB3 and is thus a mutated version of the only hitherto known enterovirus-derived CTL epitope [CVB4, P2C_1138–1146 (Varela-Calvino et al., 2004)].

Twenty-two HLA-A*01 and HLA-A*02 double-positive healthy blood donors (Supplementary Table S2 available in JGV Online) were tested for IFN-γ secretion by ELISPOT upon stimulation with the 33 predicted CVB3 epitopes. In order to detect low frequencies of CVB3-specific T cells, PBMCs were pre-sensitized. In the initial screening, the pre-sensitizing cocktail contained a set of six CVB3 peptides, a mix of HLA-matched CEF peptides (Supplementary Table S1) as well as an HLA-matched HIV peptide as a negative control (Supplementary Table S1). ELISPOTs were performed with the same set of CVB3 peptides, control peptides as well as medium and PHA controls. All positive reactions were verified in ELISPOT experiments using stimulations with only one CVB3-derived peptide. The pre-sensitization of PBMCs resulted in an amplification of pre-existing memory CTL reactions by at least 10-fold; however, no in vitro priming of CTLs was achieved as HIV stimulation did result in an IFN-γ response equal to the medium control. In total, IFN-γ secretion was observed for two HLA-A*02-restricted epitopes: GIIYIIYKL (P3A_1504–1512) and ILMNDQEVGV (P3C_1586–1595). In addition, HLA-A*01-restricted CTL reactions were observed against the epitopes MLDGHLIAFDY (RdRP_1946–1956) and YGDDVIASY (RdRP_2049–2057). The latter peptide elicited an IFN-γ response in blood donor B0788. Although the spot count index was slightly below three (mean count/mean HIV count), the peptide was included in further screening in order to validate the CTL reaction against this peptide in a higher number of blood donors. In our initial ELISPOT screening we were not able to identify any reaction against the previously published EVREKHEFL peptide (Varela-Calvino et al., 2004). This may be due to either our adaptation of the peptide sequence from CVB4 to CVB3 or it indicates a rather low immunodominance of this epitope.

Assessment of epitope immunodominance

The frequency of CTL reactions against these four peptides – indicative for their immunodominance – was assessed in a further 37 HLA-A*02- and 40 HLA-A*01-positive healthy blood donors (Supplementary Table S2). Results of positive reactions against each peptide are exemplified in Fig. 1(a). The amount of spots in the HIV control was identical to the unstimulated medium control (data not shown). A comprehensive survey of all positive ELISPOT reactions is given in Fig. 1(b). Furthermore, Supplementary Table S2 lists exact spot counts and HLA-typing of positive-reacting donors. Reactions against the GIIYIIYKL, MLDGHLIAFDY and YGDDVIASY peptides were found in 3–7 % of HLA-matching healthy blood donors. ILMNDQEVGV-specific CTL memory responses were found in approximately 25 % of all HLA-A*02-positive blood donors.

In order to assess whether the healthy blood donors who were used in our screening have previously been infected with enteroviruses, their sera were tested for neutralizing enterovirus antibodies: seven randomly chosen blood donors who exhibited no IFN-γ response in the ELISPOT screening and five donors who showed a IFN-γ response revealed neutralizing antibodies against various enterovirus strains; the results are shown in Supplementary Table S3 (available in JGV Online). Thus, we conclude that the vast majority of all tested healthy blood donors were previously exposed to enteroviruses, which is in agreement with the known epidemiological data of WHO.

Promiscuity of the identified CTL epitopes in group B enteroviruses

To analyse promiscuity of the identified CVB3 epitopes in all enteroviruses, BLAST searches (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) were performed. GIIYIIYKL, MLDGHLIAFDY and ILMNDQEVGV are present with identical sequence in more than 70 % of all group B enteroviruses (Table 1 and Supplementary Table S4 available in JGV Online). The peptide YGDDVIASY exists in only 36 % of all group B enteroviruses; however, it is also present in group C and D enteroviruses as well as in polioviruses and 5 % of all group A rhinoviruses. Thus, the four epitopes identified in the ELISPOT screening are not solely CVB3-specific, but rather represent common enterovirus group B epitopes. Regarding the monitoring of T-cell-based immune responses in patients with enterovirus myocarditis such common enterovirus epitopes are highly beneficial as different enterovirus genotypes are known to induce chronic heart disease.
Characterization of ILMNDQEVGV-specific CTL reactions

CTL reactions against ILMNDQEVGV were most dominant in the ELISPOT screening. Moreover, its sequence is present in more than 90% of all group B enteroviruses, accounting for 89.2% of all reported enteroviral infections (Table 1, Supplementary Table S3). Thus, we investigated ILMNDQEVGV-specific CTL reactions in detail. In an HLA-binding assay (Schmitz et al., 2007), we verified the high HLA-A*02-binding affinity as predicted by SYFPEITHI. ILMNDQEVGV revealed an equally good binding affinity as the allotypic HLA-A*02 peptide YLLPAIVHI (Fig. 2a).

Furthermore, a combined ILMNDQEVGV-tetramer and IFN-γ staining (Walter et al., 2005) was performed with ILMNDQEVGV-pre-sensitized PBMCs. About 1.8% of all CD8+ T cells were tetramer-positive after 12 days of pre-sensitization and two-thirds of ILMNDQEVGV-tetramer-positive cells were able to secrete IFN-γ upon the specific stimulus (Fig. 2b). Activation of PBMCs for 6 h using ILMNDQEVGV-peptide or PMA/ionomycin resulted in a downregulation of the T-cell receptor (TCR) of ILMNDQEVGV-tetramer-positive cells (Fig. 2b, top row, panels 2 and 4). In contrast, activation of PBMCs using HIV or CEF control peptides did not downregulate the TCR of ILMNDQEVGV-specific T cells (Fig. 2b, top row, panels 1 and 3). IFN-γ secretion was induced only in samples that were activated for 6 h with ILMNDQEVGV, CEF peptides or PMA/ionomycin (Fig. 2b, middle row). ILMNDQEVGV-tetramer-positive cells secreted IFN-γ only upon the specific stimulus with ILMNDQEVGV (and PMA/ionomycin). Stimulation with CEF peptides resulted in IFN-γ production only by cells that were ILMNDQEVGV-tetramer negative (Fig. 2b, bottom row).

These ILMNDQEVGV-specific CD8+ T cells exhibited a T-cell effector memory phenotype (CCR7−, CD45RA−, CD27+, data not shown). However, the ex vivo phenotype

![Image](http://vir.sgmjournals.org)
of ILMNDQEVGV-tetramer-positive T cells prior to pre-sensitization was not determinable due to their low frequency.

Cross-reactivity of ILMNDQEVGV-specific T cells

The exact sequence of ILMNDQEVGV is present in 92.4% of all group B enteroviruses for which picornain 3C sequence information is available (Table 1 and Supplementary Table S4). In all other group B enteroviruses, the sequence alters by 1 aa at different positions (listed in Supplementary Table S4). In total, only six alternative variants of ILMNDQEVGV exist in all group B enteroviruses. All these variants have a SYFPEITHI score comparable to ILMNDQEVGV. Using PBMCs that were pre-sensitized with ILMNDQEVGV, the cross-reactivity against these six variants was assessed in an IFN-γ ELISPOT (Fig. 2c). All peptides, except ILMNDQKVGV, were recognized by ILMNDQEVGV-specific T cells. Sequences with an amino acid exchange in the middle of the peptide sequence induced about 50% of the IFN-γ secretion of the original ILMNDQEVGV peptide. An amino acid exchange near the C terminus reduced the IFN-γ secretion only by 10–20%. The C-terminal amino acid serves as an anchor amino acid and is buried within the MHC molecule and thus less accessible to the TCR than amino acids in the middle of the peptide sequence. Thus, this position-specific effect of the amino acid exchange is in line with the steric orientation of the peptide within the MHC–peptide complex. Taking the cross-reactivity of ILMNDQEVGV-specific T cells into account, practically all group B enteroviruses can be recognized by these T cells.

Endogenous processing of ILMNDQEVGV

In order to verify whether ILMNDQEVGV can be processed and presented endogenously upon enteroviral infection, we infected autologous PBMCs for 1 h with m.o.i. of 10 of purified CVB3 and used these PBMCs as target T cells in an ELISPOT experiment. PBMCs pre-sensitized with ILMNDQEVGV specifically secreted IFN-γ only upon stimulation with ILMNDQEVGV or CVB3-infected autologous PBMCs (Fig. 2d), confirming endogenous processing and presentation of ILMNDQEVGV. IFN-γ secretion of PBMCs pre-sensitized with the enterovirus peptide ILMNDQEVGV in addition to HIV and CEF as control peptides was about twofold higher than that of PBMCs that were pre-sensitized with HIV and CEF control peptides only (P<0.001; data not shown). The cytolytic potential of ILMNDQEVGV-specific T cells was assessed in a Vital assay (Hermans et al., 2004). Therefore, PBMCs were pre-sensitized as described with ILMNDQEVGV, HIV and CEF peptides. ILMNDQEVGV-loaded T2 cells were used as specific target T cells, HIV-loaded T2 cells served as negative control and CEF-loaded T2 cells represented the positive control. Maximal lysis of ILMNDQEVGV-loaded T2 cells was reached in an effector to target ratio (E:T) of 10:1 (Fig. 2e). The E:T titration of CEF-loaded T2 cells did not result in a strictly sigmoidal lysis curve as the curve

<table>
<thead>
<tr>
<th>Peptide sequence</th>
<th>Position</th>
<th>Protein</th>
<th>MHC restriction</th>
<th>Frequency in reported serotype (%)</th>
<th>Frequency (%) in Healthy donors (%)</th>
<th>Syfpeithi score</th>
<th>Peptide sequence match (%)</th>
</tr>
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<tbody>
<tr>
<td>MLDGHLIAFDY</td>
<td>1946–1956</td>
<td>RNA-directed RNA polymerase</td>
<td>A*0101 (26)</td>
<td>4.8</td>
<td>4.8</td>
<td>1</td>
<td>79.2</td>
</tr>
<tr>
<td>YGDDVIASY</td>
<td>2049–2057</td>
<td>RNA-directed RNA polymerase</td>
<td>A*0101 (26)</td>
<td>3.2</td>
<td>3.2</td>
<td>1</td>
<td>79.2</td>
</tr>
<tr>
<td>GIIYIIYKL</td>
<td>1504–1512</td>
<td>Core protein P3A</td>
<td>A*0201 (27)</td>
<td>6.8</td>
<td>6.8</td>
<td>1</td>
<td>79.2</td>
</tr>
<tr>
<td>ILMNDQEVGV</td>
<td>1586–1595</td>
<td>Picornain 3C</td>
<td>A*0201 (26)</td>
<td>23.7</td>
<td>23.7</td>
<td>1</td>
<td>79.2</td>
</tr>
<tr>
<td>EV(K/R)EKHEFL</td>
<td>1138–1146</td>
<td>Core protein P2C</td>
<td>A*0201 (14)</td>
<td>5.4</td>
<td>5.4</td>
<td>1</td>
<td>79.2</td>
</tr>
<tr>
<td>EV(K/R)EKHEFL</td>
<td>1138–1146</td>
<td>Core protein P2C</td>
<td>A*0201 (14)</td>
<td>5.4</td>
<td>5.4</td>
<td>1</td>
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represents an overlay of three individual lysis curves of each CEF peptide. Finally, the cytolytic potential of ILMNDQEVGV-specific CD8\(^+\) T cells was verified in a granzyme B ELISPOT (Fig. 2f). Pre-sensitized PBMCs were found to secrete granzyme B specifically upon ILMNDQEVGV stimulation.

**Conclusions**

We have identified four HLA-A*01- and HLA-A*02-restricted enterovirus group B epitopes by IFN-\(\gamma\) ELISPot analysis of CD8\(^+\) T cells from in vivo primed individuals. ILMNDQEVGV was the most prominent CTL epitope regarding positive IFN-\(\gamma\) reactions as well as concerning its preservation in group B enteroviruses. For this peptide we showed high HLA affinity, endogenous processing of the peptide as well as a significant cytolytic capacity of ILMNDQEVGV-specific T cells. These results provide the basis for CD8\(^+\) T cell monitoring regarding the divergencies of immune reactions in enterovirus B induced diseases. It is hoped that the identified CTL epitopes may also help to explain why the immune response in some
Fig. 2. Detailed analysis of ILMNDQEVGV-specific CTL reactions. (a) ILMNDQEVGV (squares)–binding affinity was assessed in an HLA-A*02-binding assay using 1 μM of the FITC-conjugated test peptide ILK(FITC)EPVHGV. As a positive control the HLA-A*02 allotypic YLLPAIVHI (circles) was used, ILRGSVAHK (HLA-A*03, triangles) served as a negative control. (b–f) PBMCs were pre-sensitized for 12 days with ILMNDQEVGV, HIV or CEF (b) Combined tetramer IFN-γ FACS analysis of pre-sensitized PBMCs. The peptides used for 6 h stimulation prior to fluorescent staining are listed above the respective stimulations. ILMNDQEVGV-tetramer staining is indicated at the respective axis. (c) ILMNDQEVGV pre-sensitized PBMCs were stimulated with different ILMNDQEVGV variants for 6 h in an ELISPOT experiment in order to determine the T-cell cross-reactivity. The altered amino acids are enlarged in the labels. (d) Endogenous processing and presentation of ILMNDQEVGV. Pre-sensitized PBMCs of blood donors B0887 and B0974 were stimulated in an ELISPOT assay with ILMNDQEVGV or with an equal amount of autologous PBMCs. These PBMCs either were infected with m.o.i. of 10 of purified CBV3 or remained uninfected. PHA-stimulated controls are marked with an asterisk and actual spot counts are given below each well. (e) Cytolytic potential of pre-sensitized PBMCs using the Vital assay. Lysis of ILMNDQEVGV-loaded T2 cells (circles) and CEF-loaded T2 cells (squares) is depicted. E:T ratios represent the ratio of total CD8+ T cells to peptide-loaded T2 cells. (f) Granzyme B ELISPOT using pre-sensitized PBMCs of blood donor B0974. The peptides used for ELISPOT stimulation are listed above the respective stimulation, actual spot counts are given below each well.

patients can efficiently eliminate the virus from the heart during acute myocarditis, while others suffer from chronic myocarditis on the basis of a persistent infection.

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