Human CD8+ and CD4+ T Cell Memory to Lymphocytic Choriomeningitis Virus Infection

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Although cellular immunity to acute lymphocytic choriomeningitis virus (LCMV) infection has been well characterized in experimental studies in mice, the T cell response to this virus in humans is incompletely understood. Thus, we analyzed the breadths, magnitudes, and differentiation phenotypes of memory LCMV-specific CD8+ and CD4+ T cells in three human donors displaying a variety of disease outcomes after accidental needle stick injury or exposure to LCMV. Although only a small cohort of donors was analyzed at a single time point postinfection, several interesting observations were made. First, we were able to detect LCMV-specific CD8+ and CD4+ T cell responses directly ex vivo at 4 to 8 years after exposure, demonstrating the longevity of T cell memory in humans. Second, unlike in murine models of LCMV infection, we found that the breadths of memory CD8+ and CD4+ T cell responses were not significantly different from one another. Third, it seemed that the overall CD8+ T cell response was augmented with increasing severity of disease, while the LCMV-specific CD4+ T cell response magnitude was highly variable between the three different donors. Next, we found that LCMV-specific CD8+ T cells in the three donors analyzed seemed to undergo an effector memory differentiation program distinct from that of CD4+ T cells. Finally, the levels of expression of memory, costimulatory, and inhibitory receptors on CD8+ and CD4+ T cell subsets, in some instances, correlated with disease outcome. These data demonstrate for the first time LCMV-specific CD8+ and CD4+ T cells in infected humans and begin to provide new insights into memory T cell responses following an acute virus infection.

Lymphocytic choriomeningitis virus (LCMV), a single-stranded RNA virus and archetypical member of the family Arenaviridae, is a human pathogen maintained in a rodent host reservoir. Infection outcomes can range from subclinical disease to aseptic meningitis, encephalitis, life-threatening infections in immunosuppressed individuals, and severe congenital defects (5, 6, 9, 19). Despite the pathogenic nature of LCMV, there are no U.S. Food and Drug Administration-approved vaccines available. Moreover, antiviral therapy is limited to the use of the guanosine analogue ribavirin, which can lead to adverse side effects such as thrombocytosis, severe anemia, and birth defects (18, 37). Animal models of LCMV infection have clearly demonstrated that virus-specific CD8+ and CD4+ T cells play a pivotal role in viral clearance and protection (17, 20, 26, 27, 58, 59, 61). However, to date, human T cell responses to LCMV have not been characterized in detail.

We have begun to understand human LCMV-specific T cell responses by recent work with HLA transgenic mice, identifying HLA-restricted CD8+ and CD4+ T cell epitopes (10, 31, 32). Immunization of mice with HLA-restricted CD8+ T cell epitopes is sufficient to confer protection against subsequent infection with LCMV in HLA transgenic mice (10). However, the degree of overlap between T cell epitopes identified in HLA transgenic mouse models and humans is incomplete (30, 55). Thus, whether the epitopes defined in the HLA transgenic model are useful to characterize human responses remains to be ascertained experimentally.

Human virus-specific CD8+ T cell responses have been extensively characterized in the context of persistent virus infections, including infections with cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis C virus (HCV), and human immunodeficiency virus type 1 (HIV-1). By examining the expression profiles of surface markers such as CD45RA, CCR7, CD62L, CD28, and CD27, human CD8+ T cells from different persistent virus infections could be categorized into phenotypically distinct memory populations (2, 12, 49). More recently, there have been several investigations examining the differentiation phenotypes of antiviral CD8+ T cells responding to live-attenuated viral vaccines, such as those for smallpox virus (Dryvax) and yellow fever virus (YFV)-17D (1, 13, 38). These
studies have shown that memory CD8+ T cells generated after an acute infection with live-attenuated vaccines exhibit phenotypic and functional characteristics that are distinct from those described for human CD8+ T cells specific for persistent viruses (1, 38). Importantly, effector memory CD45RA+CCR7−CD8+ T cells from persistent infections have been defined as terminally differentiated, while after acute infections, CD45RA-expressing CD8+ T cells exhibit a robust proliferative capacity. Although a great deal of knowledge characterizing Dryvax- and YFV-17D-specific CD8+ T cells has been gained, results from these studies are difficult to generalize to other acute virus infections because of the attenuation state of the virus inoculum and a lack of data in regard to virus-specific CD4+ T cells. Other acute virus infections have also been studied, such as hantavirus and influenza A virus (23, 56); however, these are not ideal experimental systems because exposure to virus and virus reencounter cannot be controlled.

In the present study, we report the identification of LCMV-specific CD8+ and CD4+ T cell epitopes restricted by various HLA molecules. In the case of HLA molecules previously investigated in HLA transgenic mouse models, we report an excellent overlap between epitopes recognized in humans and HLA transgenic mice. Utilizing these T cell epitopes, we characterized the breadths, magnitudes, and differentiation phenotypes of the memory CD8+ and CD4+ T cell responses generated after acute LCMV infection in a small cohort of human donors who displayed a range of disease outcomes.

MATERIALS AND METHODS

Ethics statement. Research conducted for this study was performed in accordance with approvals from the Institutional Review Board at the La Jolla Institute for Allergy and Immunology. All participants provided written informed consent prior to participation in the study.

Study subjects and PBMC samples. Healthy volunteers (>18 years of age) with a history of accidental needle stick injury or exposure to LCMV were recruited to the study following informed consent. Seropositivity was confirmed with a history of accidental needle stick injury or exposure to LCMV were studied, such as hantavirus and influenza A virus (23, 56); however, these are not ideal experimental systems because exposure to virus and virus reencounter cannot be controlled.

In the present study, we report the identification of LCMV-specific CD8+ and CD4+ T cell epitopes restricted by various HLA molecules. In the case of HLA molecules previously investigated in HLA transgenic mouse models, we report an excellent overlap between epitopes recognized in humans and HLA transgenic mice. Utilizing these T cell epitopes, we characterized the breadths, magnitudes, and differentiation phenotypes of the memory CD8+ and CD4+ T cell responses generated after acute LCMV infection in a small cohort of human donors who displayed a range of disease outcomes.

RESULTS

Breath and magnitude of LCMV-specific memory CD8+ T cell responses in a small cohort of individuals with resolved acute LCMV infection. We examined responses in three subjects who displayed various degrees of symptoms after accidental needle stick injury or exposure with two different strains of LCMV (Table 1). Subject AD108 was infected with the Arm-
strong clone 53b strain of LCMV, while subjects AD123 and AD124 were infected with the clone 13 strain. All three subjects displayed LCMV-specific serum IgG antibodies but were negative for LCMV-specific serum IgM (data not shown). The specificity of the serology assay was confirmed by testing sera from a total of 30 subjects with no history of LCMV exposure. All 30 subjects were negative for both LCMV-specific IgG and IgM antibodies. AD108 was clinically unawares of his infection, thus allowing us to study an asymptomatic acute LCMV infection. AD123 and AD124 were both symptomatic, and infection resulted in either a flu-like illness or aseptic meningitis, respectively. To investigate the breadth and magnitude of LCMV-infection, peptides derived from the GPC and NP predicted to bind to the donors’ specific haplotypes and an HLA-A*0201-restricted epitope, Z49-58, previously identified in HLA transgenic animals, were synthesized. PBMC isolated from the three donors were stimulated with the candidate peptides, and positive peptides were identified on the basis of their ability to stimulate IFN-γ production from CD8+ T cells in an ELISPOT assay.

In total, nine unique CD8+ T cell epitopes were defined from the three individuals. CD8+ T cells specific for the GPC, NP, and Z proteins were detected, indicating that all three viral proteins are immunogenic in humans (Table 2 and Fig. 1). As a specificity control, CD8+ T cells isolated from three LCMV-seronegative individuals who expressed the same HLA class I molecules as our donor subjects (representative data are shown in Fig. 1) were also examined. Because the defined epitopes were restricted by 6 different HLA class I alleles, a total of 18 HLA-matched, LCMV seronegative PBMC (3 donors/allele) were tested to ensure that the epitope-specific responses were truly LCMV specific. None of the epitopes were recognized by CD8+ T cells isolated from the seronegative donors, thus confirming the specificity of the defined epitopes.

Since three or four CD8+ T cell epitopes were identified in each individual, disease severity and/or the infecting virus strain did not appear to affect the overall breadth of the memory CD8+ T cell response. However, the magnitude of the total CD8+ T cell response seemed to correlate with increased clinical severity, with the lowest responses detected in AD108 (total of 228 net SFC/10^6 cells) and significantly higher responses observed in AD123 and AD124 (totals of 734 and 863 net SFC/10^6 cells, respectively) (P < 0.05).

As shown in Table 2, the HLA restriction of the newly defined LCMV-specific CD8+ T cell populations, inferred by the bioinformatic predictions, was further supported by experiments measuring epitope binding to purified HLA molecules in vitro. In 7 out of 10 cases, the epitope bound the relevant HLA molecule with high affinity (1 to 100 nM range), and in the 3 remaining cases it did so with weak to intermediate affinity (10 to 10,000 nM range). Two CD8+ T cell epitopes (GPC447-455 and NP246-254) were recognized in more than one donor and were restricted by distinct MHC class I types. An overlapping

### Table 1. Characteristics of LCMV-seropositive donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Sex</th>
<th>HLA class I (DRB1)</th>
<th>HLA class II</th>
<th>LCMV strain</th>
<th>Yr of infection</th>
<th>Route of infection</th>
<th>Symptoms and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD108</td>
<td>Male</td>
<td>A<em>2402 A</em>3201 B<em>0702 B</em>1501</td>
<td>*1101 *1401</td>
<td>Armstrong clone 53b Clone 13</td>
<td>2003</td>
<td>Unknown</td>
<td>Flu-like illness and bacterial infection, 60 days</td>
</tr>
<tr>
<td>AD123</td>
<td>Male</td>
<td>A<em>0201 B</em>1401 B*4402</td>
<td>*0701 *0801</td>
<td>Clone 13</td>
<td>2001</td>
<td>Leg needle stick</td>
<td>Aseptic meningitis, 4 to 5 days</td>
</tr>
<tr>
<td>AD124</td>
<td>Male</td>
<td>A<em>0201 B</em>1402 B<em>4801 B</em>5401</td>
<td>*0803 *0901</td>
<td>Clone 13</td>
<td>2002</td>
<td>Eye</td>
<td>None</td>
</tr>
</tbody>
</table>

### Table 2. Properties of LCMV-derived HLA class I-restricted CD8+ T cell epitopes

<table>
<thead>
<tr>
<th>Donor</th>
<th>Epitope</th>
<th>Peptide sequence</th>
<th>ELISPOT assay result</th>
<th>Predicted HLA restriction</th>
<th>Measured binding affinity (nM)</th>
<th>Restriction (tetramer staining)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD108</td>
<td>GPC38-46</td>
<td>FATCGIFAL</td>
<td>63</td>
<td>B*0702</td>
<td>6,230</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>GPC447-455</td>
<td>YLVSIFLH</td>
<td>46</td>
<td>B*1501</td>
<td>6,920</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NP246-254</td>
<td>AAVKAGAAL</td>
<td>46</td>
<td>B*0702</td>
<td>9,8</td>
<td>B*0702</td>
</tr>
<tr>
<td></td>
<td>NP414-422</td>
<td>KQFKQDSKY</td>
<td>73</td>
<td>B*1501</td>
<td>13</td>
<td>B*1501</td>
</tr>
<tr>
<td>AD123</td>
<td>GPC447-455</td>
<td>YLVSIFLH</td>
<td>172</td>
<td>A*0201</td>
<td>35</td>
<td>A*0201</td>
</tr>
<tr>
<td></td>
<td>NP45-53</td>
<td>SEVSNVQRI</td>
<td>84</td>
<td>B*4402</td>
<td>12</td>
<td>B*4402</td>
</tr>
<tr>
<td></td>
<td>NP69-77</td>
<td>SLQTVHSL</td>
<td>348</td>
<td>A*0201</td>
<td>53</td>
<td>A*0201</td>
</tr>
<tr>
<td></td>
<td>Z49-58</td>
<td>YLCRHCLNL</td>
<td>130</td>
<td>A*0201</td>
<td>3.9</td>
<td>A*0201</td>
</tr>
<tr>
<td>AD124</td>
<td>GPC10-18</td>
<td>ALPHHIDEV</td>
<td>413</td>
<td>A*0201</td>
<td>57</td>
<td>A*0201</td>
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<tr>
<td></td>
<td>GPC11-19</td>
<td>LPHHIDEVI</td>
<td>151</td>
<td>B*5401</td>
<td>1,400</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NP246-254</td>
<td>AAVKAGAAL</td>
<td>299</td>
<td>B*4802</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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* a: Peptide position within LCMV strain Armstrong clone 53b GPC, NP, or Z protein.
* b: Number of net SFC per 10^6 purified CD8+ T cells. Data are averaged from two independent experiments.
* c: Peptides were tested for binding to their predicted HLA class I allele. ND, binding was not determined because the purified HLA was not available.
* d: Restriction was determined by HLA class I tetramer staining. ND, tetramer staining was not performed.
epitope sequence was also defined in AD124, with GPC_{10-18} binding to HLA-A*0201 and GPC_{11-19} to HLA-B*5401.

LCMV-specific memory CD4^+ T cell responses in same three individuals with resolved acute infection. CD4^+ T cells targeted both the GPC and NP, with three unique epitopes derived from the GPC and nine from the NP (Table 3 and Fig. 2). A single epitope (NP_{521-533}), originally defined in HLA-DRB1*0101 transgenic mice (33), was also recognized by AD123 (Table 4). Epitope-specific responses for AD108 and AD123 were not detectable in HLA-matched, LCMV-seronegative donors (representative data are given in Fig. 2). The overall breadth of the LCMV-specific memory CD4^+ T cell response did not appear to correlate with disease severity and/or the infecting virus strain. Unlike the case for the LCMV-specific CD8^+ T cell response, the magnitude of the total CD4^+ T cell response was highly variable between the three different donors examined. The total net SFC/10^6 CD4^+ T cells for AD108, AD123, and AD124 were 121, 1,279, and 100, respectively.

The restricting alleles of the LCMV-specific CD4^+ T cell populations inferred by the predicted binding affinities were further supported by the measured binding affinities (Table 3). In 7 out of 14 cases, the epitope bound the relevant HLA molecule with high affinity (1 to 100 nM range), and in the 7 remaining cases it did so with weak to intermediate affinity (100 to 10,000 nM range). Two GPC-specific epitopes (GPC_{66-80} and GPC_{71-85}) were recognized in more than one donor and were restricted by different HLA-DRB1 molecules. GPC_{66-80} and GPC_{71-85} both bound to HLA-DRB1*0701 in AD123 and to HLA-DRB1*0901 in AD124.

FIG. 1. Direct ex vivo detection of LCMV-specific memory CD8^+ T cell responses. Purified CD8^+ T cells from either the LCMV-seropositive donors (black bars) or a representative HLA-matched, LCMV-seronegative donor (white bars) were incubated with 10 μg/ml peptide, after which the number of IFN-γ-producing cells was enumerated in an ELISPOT assay. Shown are the average net SFC/10^6 CD8^+ T cells after which the number of IFN-γ-producing cells was enumerated in an ELISPOT assay. Shown are the average net SFC/10^6 CD8^+ T cells for specific epitopes with binding affinities to the predicted HLA allele of <100 nM (Tables 2 and 3). Because the frequencies of LCMV-specific CD8^+ and CD4^+ T cell populations detected by ELISPOT assay in the three individuals were relatively low, we employed tetramer staining followed by a magnetic bead enrichment technique (see Materials and Methods) to increase the sensitivity of detection (4, 45).

The tetramer staining experiments confirmed HLA restriction of seven CD8^+ and four CD4^+ T cell responses (representative data are given in Fig. 3). We were able to detect LCMV-specific T cell responses at frequencies 30- to 600-fold above background. For some CD8^+ and CD4^+ T cell populations, we were unable to confirm the HLA restriction inferred on the basis of the HLA predictions (Tables 2 and 3), because purified HLAs for binding assays and/or tetramer preparations were not available. Only a small number of background-level tetramer-positive cells were detected with the LCMV-specific tetramers in the control HLA-matched, LCMV-seronegative individuals (Fig. 3), thus confirming that tetramer specificity was derived from the LCMV peptide and HLA molecule combination.

Phenotypic characterization of the LCMV-specific memory CD8^+ T cells. Next, we assessed the differences between effector and central memory T cells using CD45RA, a transmembrane tyrosine phosphatase reexpressed on late-differentiated CD8^+ T cells (12), and CCR7, a chemokine receptor that controls homing to secondary lymphoid organs (49). As shown in Fig. 4, we found that LCMV-specific tetramer-positive CD8^+ T cells consisted predominantly of CD45RA^+CCR7^+ effector memory cells in the three different donors analyzed. We also analyzed the expression of CD62L, an L-selectin lymph node homing molecule. LCMV-specific CD8^+ T cell subsets consisted mainly of CD62L^{low} effector memory cells but also contained CD62L^{hi} central memory cells (Fig. 4), particularly in subjects AD108 and AD124. Interestingly, the majority of tetramer-positive CD8^+ T cells isolated from AD123 consistently displayed CD45RA^{low} and CD62L^{low} ex-
pression compared with the other two donors (medians of 34% CD45RA+ and 12% CD62L+). In most instances, we found similar expression patterns of CD45RA, CCR7, and CD62L in both the tetramer-positive CD8+ T cells and the total CD8+ T cell population within the same donor.

To further determine the phenotypic nature of the LCMV-specific memory CD8+ T cells, we examined the coexpression of CD127 (interleukin-7 receptor alpha [IL-7Rα]), which marks precursors destined to differentiate into long-lived memory cells (25), and the chemokine receptor CCR5, which is associated with activated memory cells (46). We found that the majority of tetramer-positive CD8+ T cells reexpressed CD127 in subject AD108 (median of 69% CD127+), whereas CD127 was reexpressed at progressively lower levels in AD123 and AD124 (medians of 22% and 10% CD127+, respectively). Bimodal expression patterns were observed for CCR5 in all three donors.

**Characterization of the LCMV-specific CD8+ T cells in terms of expression of costimulation and inhibitory receptors.** To assess the ability of the memory CD8+ T cells to respond to costimulatory signals, we studied the expression of CD27 and CD28. These costimulatory receptors have also been used to phenotypically differentiate distinct memory CD8+ T cell subsets following persistent virus infections (2). We found that tetramer-positive CD8+ T cells from AD108 and AD123 showed uniformly high expression of CD27 and heterogeneous expression of CD28, while only ~30% of tetramer-positive CD8+ T cells from AD124 expressed both CD27 and CD28.

We then examined the expression of two inhibitory receptors, programmed death 1 (PD1) and lymphocyte activation gene 3 (LAG3), that both negatively regulate T cell activation and proliferation when upregulated transiently in acute LCMV Armstrong infection and long term in clone 13 infection of mice (3, 7). Recently, it was shown that blockade of both PD1 and LAG3 improved CD8+ T cell responses and reduced the viral load in LCMV clone 13-infected mice (7). We found that tetramer-positive CD8+ T cells from all three donors did not express PD1, which is consistent with a resolved acute infection and clearance of virus. In contrast, we observed upregulated expression of LAG3 in the three donors (medians of 6% for AD108, 19% for AD123, and 4% for AD124). Interestingly, a high degree of variability was observed when examining the expression of LAG3 in other tetramer-positive CD8+ T cell populations from AD123. Ten percent of HLA-A*0201

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**TABLE 4. Summary of overlap between HLA class I- and II-restricted LCMV T cell epitope recognition in human and HLA transgenic mouse systems.**

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Sequence</th>
<th>Human system</th>
<th>Transgenic mouse system</th>
<th>Identification</th>
<th>Reference(s)(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPC10-18</td>
<td>ALPHIDEV</td>
<td>Y</td>
<td>Y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>GPC17-19</td>
<td>LPHIDEV</td>
<td>Y</td>
<td>NT</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>GPC34-43</td>
<td>AVYNFATCGI</td>
<td>NT</td>
<td>Y</td>
<td>10, 31</td>
<td></td>
</tr>
<tr>
<td>GPC38-46</td>
<td>FATCIGIFAL</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPC112-120</td>
<td>FTNDISISH</td>
<td>N</td>
<td>Y</td>
<td>31</td>
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<tr>
<td>GPC46-55</td>
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<td>NT</td>
<td>Y</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>GPC447-455</td>
<td>YLSIFILHL</td>
<td>Y</td>
<td>Y</td>
<td>10</td>
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</tr>
<tr>
<td>NP45-53</td>
<td>SESVNVORI</td>
<td>Y</td>
<td>NT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP10-77</td>
<td>SLNTOVHSL</td>
<td>Y</td>
<td>Y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>NP246-254</td>
<td>AAVKAGAAL</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP414-422</td>
<td>KQKFODSKY</td>
<td>Y</td>
<td>NT</td>
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<tr>
<td>NP521-535</td>
<td>MDCIFESASKRLP</td>
<td>Y</td>
<td>Y</td>
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<td>Z23-33</td>
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<td>NT</td>
<td>Y</td>
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<tr>
<td>Z49-58</td>
<td>YLCHRCHNLY</td>
<td>Y</td>
<td>10, 31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) Y, epitope was identified; N, epitope was not identified. Class I epitope recognition in HLA transgenic mice was examined following inoculation of mice with the LCMV strain Armstrong (10) or recombinant VACV (rVACV) expressing LCMV GPC, NP, or Z (31). Class II epitope recognition was examined after priming mice with an rVACV expressing one of the LCMV proteins followed by boosting with LCMV-specific peptide pools (33). Epitope-specific T cell responses were measured by IFN-γ ELISPOT assays. NT, not tested because the epitope sequence was not predicted to bind to the donor's HLA molecules or because HLA transgenic mice were not available.

(b) Reference(s) for epitope identification in HLA transgenic mice.
NP_{69-77}, 23% of HLA-A*0201 GPC_{447-455}, and 42% of HLA-B*4402 NP_{45-53} were positive for LAG3 (median of 19% LAG3^+ cells) (data not shown).

Phenotypic characterization of the LCMV-specific memory CD4^+ T cells. Our understanding of CD4^+ T cell differentiation following acute virus infection in humans remains unclear. To begin to fill this knowledge gap, we applied the tetramer enrichment technique to phenotypically characterize LCMV-specific CD4^+ T cell populations isolated from AD108 and AD123. We were unable to phenotype CD4^+ T cells from AD124, as the MHC class II molecules DRB1*0803 and DRB1*0901 were not available for tetramer preparation. The expression of markers used to dissect the CD8^+ T cell populations was also used to characterize the LCMV-specific CD4^+ T cells.

Overall, we observed two key differences between the

![FIG. 2. Direct ex vivo detection of LCMV-specific memory CD4^+ T cell responses. PBMC enriched for CD4^+ T cells from either the LCMV-seropositive donors (black bars) or a representative HLA-matched, LCMV-seronegative donor (white bars) were incubated with 10 μg/ml peptide. The number of IFN-γ-producing cells was enumerated in an ELISPOT assay. Shown are the average net SFC/10^6 CD4^+ T cells for AD108 (A), AD123 (B), and AD124 (C) in response to the defined epitopes from at least two independent experiments. An HLA-matched, LCMV-seronegative donor for AD124 was not available. The dashed lines indicate the threshold for peptide positivity (≥20 net SFC/10^6 cells). For each individual experiment, samples were tested in triplicate. Error bars indicate SEM.](http://jvi.asm.org/Downloaded from)
tetramer-positive CD8+ and CD4+ T cell populations analyzed. First, we found that tetramer-positive CD4+ T cells consisted predominantly of CD45RA+/CCR7+CD62L+ effector memory cells (Fig. 5). Second, when examining CD27 and CD28 expression, we found that the tetramer-positive CD4+ T cells uniformly expressed high levels of both costimulatory receptors. Expression of CD127 was divergent between the two donors analyzed. Greater than 70% of tetramer-positive CD4+ T cells isolated from AD108 reexpressed high levels of CD127, whereas the majority of tetramer-positive CD4+ T cells from AD123 expressed CD127 at only low to moderate levels (median of 16% CD127+). Interestingly, similar to the case for LCMV-specific CD8+ T cells from AD123, LAG3 expression was also upregulated on tetramer-positive CD4+ T cells in this donor. This phenotype was seen on other tetramer-positive CD4+ T cell populations isolated from AD123 (median of 27% LAG3+) (data not shown). Both CCR5 and PD1 were not expressed on any of the CD4+ T cell populations analyzed.

FIG. 3. HLA restriction of LCMV-specific CD8+ and CD4+ T cell responses using MHC tetramers. PBMC from AD108, AD123, or HLA-matched, seronegative individuals were stained with MHC class I tetramers (A) or class II tetramers (B), and tetramer-positive cells were isolated following magnetic bead enrichment. Plots are gated on either CD3+CD8+ (A) or CD3+CD4+ (B) lymphocytes, and the numbers indicate the total number of tetramer-positive cells isolated from each donor’s PBMC.

FIG. 4. Memory phenotype of LCMV-specific CD8+ T cells. PBMC from donors AD108, AD123, and AD124 were stained with the MHC class I tetramers HLA-B*1501 NP414-422, HLA-A*0201 Z49-58, and HLA-A*0201 GPC10-18, respectively. Tetramer-positive cells were enriched following incubation with magnetic beads. There was a 10-fold reduction in the number of tetramer-positive CD8+ T cells isolated from AD124 when examining CD127, CCR5, CCR7, CD28, LAG3, and PD1 expression, as fewer PBMC were initially used. Plots are gated on either total CD3+CD8+ T cells (black background) or LCMV-specific CD3+CD8+ T cells (red dots). The numbers represent the percentages of tetramer-positive CD3+CD8+ T cells in the gate.
The present study represents the first characterization of human memory T cell responses to LCMV in the setting of accidental needle stick injury or exposure with different strains of LCMV in three individuals who displayed distinct disease outcomes. Responses against a total of 9 unique CD8\(^+\) and 12 CD4\(^+\) T cell epitopes were detected directly \textit{ex vivo} at 4 to 8 years after LCMV exposure. We were able to rapidly identify these T cell epitopes, as HLA binding predictions are increasingly more accurate and IFN-\(\gamma\) ELISPOT assays allow quick screening of a large number of peptides. Most importantly, the integration of bioinformatic approaches with cellular immune assays might lead to the development of optimized vaccines and drugs tailored to personalized prevention and treatment of virus infection. Thus, with the advent of epitope identification in humans, personalized medicine is becoming a reality.

Despite our findings, the small number of individuals available to us for study and the significant time elapsed from infection dictate that these evaluations should be interpreted with caution. The data trends and conclusions suggested in this study would be strengthened by increasing the number of subjects and/or by analysis of the same three subjects at multiple time points to enable documentation of differentiation changes of the defined T cell populations. Unfortunately, we have been constrained by the wide geographic distribution of our donors and thus are unable to obtain repeat PBMC donations from the same three subjects. Further studies with additional donors and time points postinfection would be required to more firmly establish the observations reported here. These are areas of future research.

Although the donor cohort size was small, we detected a high degree of overlap in the epitopes recognized in infected humans and in previous studies utilizing HLA transgenic mice. Of the 5 HLA-A*0201-restricted LCMV-specific CD8\(^+\) T cell epitopes originally defined in HLA-A*0201 transgenic mice, 4 were also recognized by HLA-A*0201-positive donors in this study, thus demonstrating an 80% concordance rate between humans and mice. The facts that multiple HLA-A*0201-positive donors recognized the HLA-A*0201-restricted CD8\(^+\) T cell epitopes and that immunization with these epitopes protected mice against LCMV challenge further validate their potential use as vaccine candidates. The high rate of concordance between the two systems underscores the importance of HLA transgenic mice as a suitable model for identifying human T cell epitopes from small RNA viruses, such as LCMV. HLA transgenic mice also serve as a preliminary probe for identifying T cell epitopes from viruses that infrequently infect humans and that could be viewed as potential bioterrorist threats.

In the three donors analyzed, we found that the breadths of the memory CD8\(^+\) and CD4\(^+\) T cell responses did not significantly differ from one another and were not influenced by disease severity and/or the infecting LCMV strain. In contrast, the overall magnitudes of memory CD8\(^+\) and CD4\(^+\) T cell responses varied between the different donors. Compared to the total CD8\(^+\) T cell response magnitude, the total CD4\(^+\) T cell response was similar in AD108, higher in AD123, and lower in AD124. These findings were interesting, as they differ from what was previously observed in murine models of LCMV infection and also in the case of other acute viral infections in humans. Thus, it appears that different viruses might induce qualitatively different T cell responses. In murine models of LCMV, studies have demonstrated that a broader repertoire of CD8\(^+\) T cell responses exists after an acute LCMV infection (16, 32). Murine models have also demon-

**DISCUSSION**

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strated major differences in the magnitudes of memory CD8+ and CD4+ T cell responses. Memory is stable in CD8+ T cell responses, while the levels of memory CD4+ T cells are 20-fold lower and gradually decline (15, 24). Our findings also differ from those for other acute viral infections in humans, including severe acute respiratory syndrome (SARS) coronavirus (35) and vaccinia virus (VACV) (38), where memory CD8+ T cell responses are more frequent and greater in magnitude than CD4+ T cell responses (10, 31).

We also found that the total LCMV-specific CD8+ T cell response was augmented with increasing severity of disease, while the total CD4+ T cell response was highly variable between the different donors. This variability could be due to the small size of the donor cohort analyzed. These findings are in agreement with studies on other acute viral infections in humans, including Sin Nombre virus and dengue virus, which have demonstrated that elevated levels of virus-specific CD8+ T cells correlate with heightened disease severity (29). Infection with Sin Nombre virus, a hantavirus that causes fulminant life-threatening disease, results in high frequencies of tetramer-specific CD8+ T cells in patients with severe hantavirus pulmonary syndrome compared to those in patients with moderate disease (29, 39, 40). Similarly, higher frequencies of circulating dengue virus-specific CD8+ T cells are seen in patients with acute secondary dengue virus infection (40). Thus, whether disease outcome correlates with the virus-specific T cell response seems to depend on the infecting virus, and the fine balance between protective immunity and immunopathology. However, in our case, we have been able to study only infections resolved some time previously, so it is difficult to determine the importance of these cells in the development of acute infection and pathogenesis.

To our knowledge, this is one of the first studies where the differentiation phenotypes of human LCMV-specific CD8+ and CD4+ T cells have been compared following an acute, live nonattenuated virus infection in the absence of antigenic reencounter (14, 28, 36, 50). When examining the differences between effector memory and central memory on the basis of CD45RA, CCR7, and CD62L expression patterns, we found that LCMV-specific CD8+ T cells had a predominantly effector memory phenotype, with the majority of tetramer-positive CD8+ T cells reexpressing CD45RA. This CD45RA+ effector memory phenotype confirms what was previously observed in vaccinees acutely infected with either Dryvax or YFV-17D (1, 13, 38, 44) and expands these observations to LCMV infection. Interestingly, this general observation held true for the three different donors, despite dramatic differences in the severity of disease associated with the infection. Interestingly, we noted that in some instances MHC-peptide tetramer staining detected a 5-fold-greater number of LCMV-specific T cells than was demonstrated to produce IFN-γ (HLA-B*1501-restricted NP414-422 and HLA-A*0201-restricted Z49-58). It has been previously reported that in some cases there is a 1:1 correspondence between the number of antigen-specific T cells detected by IFN-γ ELISPOT and tetramer staining assays, while in other cases this correspondence is not observed. As noted by Murali-Krishna et al. (42), in BALB/c mice infected with LCMV, both IFN-γ ELISPOT and tetramer staining detected approximately 50% of CD8+ T cells recognizing the immunodominant epitope NP116-126. However, in LCMV-infected C57BL/6 mice, 33% of CD8+ T cells recognizing the immunodominant H-2D^d-restricted NP396-404 epitope were detected by IFN-γ ELISPOT, while only 23% of CD8+ T cells bound the H-2D^d NP396-404 tetramer. In humans, there is also a correlation between the number of antigen-specific T cells detected by IFN-γ ELISPOT and tetramer staining assays; however, the ratio is usually not 1:1. In agreement with our data, in HIV-infected individuals, tetramer staining detected approximately a 5- to 10-fold-greater number of CD8+ T cells recognizing HLA-A02-restricted Gag and Pol-specific epitopes than were demonstrated to produce IFN-γ (22, 54). A larger number of antigen-specific T cells could be detected by tetramer staining assays, because tetramer-positive T cells might be producing other cytokines or no cytokines at all. Another possibility is that a portion of tetramer-positive T cells are no longer able to proliferate and are undergoing apoptosis.

The availability of novel MHC class II tetramers allowed us for the first time to evaluate the memory phenotype of virus-specific CD4+ T cells resulting from an acute viral infection in humans. Importantly, we demonstrated that LCMV-specific CD4+ T cells had an effector memory phenotype different from that of CD8+ T cells. The majority of tetramer-positive CD4+ T cells lacked CD45RA expression. The lack of CD45RA expression has been suggested to reflect a possibly less mature or differentiated effector memory phenotype (48, 53). Thus, it seems that following acute LCMV infection in humans, CD4+ T cells have a less mature memory phenotype than CD8+ T cells. Considering the phenotypic differences between CD8+ and CD4+ T cell subsets unearthed in the present study, this suggests that a more thorough examination of virus-specific CD4+ T cells is needed in future studies.

The majority of LCMV-specific CD8+ and CD4+ T cells expressed high levels of both CD28 and CD45RA (1), while CD4+ T cells expressed high levels of both CD28 and CD27, which is consistent with the early differentiation phenotype also seen in influenza virus-infected individuals and Dryvax and YFV17D vaccinees (1, 23, 38). We expanded on these findings by demonstrating that the level of CD28 and CD27 expression appears to correlate with disease outcome. Specifically, we found that the tetramer-positive CD8+ T cells from AD124 were predominantly CD28+ CD27+, suggesting a late differentiated phenotype also seen in CMV infections (2). Interestingly, early CD28+ CD27+ differentiated antiviral T cells have been associated with protective immunity (47), suggesting that a more severe disease outcome, as seen in AD124, might be related to the quality of the antiviral CD8+ T cell response.

Finally, CD127 and LAG3 expression also correlated with clinical manifestation. CD127 is reexpressed on antiviral CD8+ T cells in resolved infections (8, 25) but is not present at significant levels on CMV- and EBV-specific CD8+ T cells, demonstrating that persistent viral antigen suppresses CD127 expression (21, 34, 57). In our study, CD127 was expressed at high levels on LCMV-specific CD8+ and CD4+ T cells in the asymptomatic AD108 donor, while CD127 expression rapidly declined with increased disease severity in AD123 and AD124. LAG3 expression was also upregulated on all tetramer-positive T cell subsets examined, especially CD8+ and CD4+ T cells in donor AD123. Similarly, the upregulation of CD127 on HBV-specific CD8+ T cells correlated with a loss of PD1 expression (8). These results suggest a reciprocal relationship between CD127 and inhibitory receptor expression.
In conclusion, these data collectively demonstrate for the first time that memory CD8$^+$ and CD4$^+$ T cells can be identified in infected humans. Most notably, the rapid identification of T cell epitopes from infected humans suggests that personalized prevention and treatment of virus infection are possible. In addition, this study not only confirms previous findings with live attenuated viral vaccines but also begins to show that disease outcome correlates with both the magnitude of the memory CD8$^+$ T cell response and the phenotypes of both LCMV-specific CD8$^+$ and CD4$^+$ T cells. These data suggest that a closer examination of LCMV-specific CD8$^+$ and CD4$^+$ T cells with a larger donor cohort is needed in future studies.

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