Sustained CD8+ T-Cell Responses Induced after Acute Parvovirus B19 Infection in Humans

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Murine models have suggested that CD8+ T-cell responses peak early in acute viral infections and are not sustained, but no evidence for humans has been available. To address this, we longitudinally analyzed the CD8+ T-cell response to human parvovirus B19 in acutely infected individuals. We observed striking CD8+ T-cell responses, which were sustained or even increased over many months after the resolution of acute disease, indicating that CD8+ T cells may play a prominent role in the control of parvovirus B19 and other acute viral infections of humans, including potentially those generated by live vaccines.

The emergence of new tools for the ex vivo analysis of cellular immune responses, especially CD8+ T-cell responses, has revealed an important role for such cells in a range of viral infections. In studies with mice, most work on acute infection has been focused on influenza and lymphocytic choriomeningitis virus (6, 11, 29). However, very little is known about CD8+ T-cell responses in acute infections in humans; most work has focused on latent and persistent infections, such as human immunodeficiency virus, cytomegalovirus (CMV), Epstein-Barr virus, and hepatitis B and C virus (1, 16, 17) infections. In infections which are truly cleared, such as influenza, responses may return to a low-level resting memory state (20). In contrast, in infections with very-low-level persistence, such as CMV infection, strong immune responses may be sustained, and indeed, may increase over time (12, 13). Such responses typically possess “mature” effector characteristics indicative of repetitive antigen exposure (2, 26). Parvovirus B19 (B19) is a common virus with significant pathology (7). As B19 is regarded as a typical “hit-and-run” virus, the humoral response plays a well-documented role for viral neutralization, but there is also evidence that low-level persistence can occur in certain cases (21, 27). Cellular immune responses have also recently been described, both CD4+ proliferative- and CD8+ cytotoxic-T-cell responses, with one HLA-B35-restricted epitope characterized so far (4, 5, 28). The small B19 genome is very stable and encodes only three major proteins, which makes it suitable for extensive study without compromise due to incoming antigenic variability. Here, we describe the first assessment of the variability. Here, we describe the first assessment of the
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PBMC was depleted of CD8⁺ T cells by using microbeads (Miltenyi Biotec, Gladbach, Germany). Nonamer-mediated cytotoxicity was tested by 51Cr-release assay (22, 25). HLA restrictions were estimated by using the BIMAS algorithm (http://bimas.cit.nih.gov) and T2-cell assays (14) and by matching single HLA alleles of targets and effectors in ⁵¹Cr experiments.

All individuals showed normal mitogen-induced IFN-γ responses and proliferation in vitro (data not shown). Fever, rashes, and fatigue resolved within 6 weeks. Responses to 8 of
the 14 NS pools and 1 of the 6 VP2 pools were shown. No responses to the VP1ur pools were shown. All individuals responded to NS, whereas a VP2 response was present in only two individuals. Responses peaked at around 1 year in S1, S2, and S3, with a decline at about 2 years, whereas S4 showed a more rapid course, with a peak at 15 weeks and a decline at 1 year (Fig. 1). S5 was followed for 48 weeks with stable response levels. In S2, IgM antibodies were detected for more than 90

![Graph](image)

**FIG. 1—Continued.**

<table>
<thead>
<tr>
<th>Peptide name&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amino acid sequence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HLA restriction</th>
<th>Specific lysis (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Response after CD8&lt;sup&gt;d&lt;/sup&gt; depletion (%)&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (9) 102-GLF</td>
<td>GLFNNVLYH</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>55</td>
<td>31</td>
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<tr>
<td>NS (9) 247-SSH</td>
<td>SSSHSGFOI</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>65</td>
<td>0</td>
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<tr>
<td>NS (9) 276-LLH</td>
<td>LLHTDFEQVM</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>NS (9) 277-LHT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>LHTDFEQVM</td>
<td>A*02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>NS (9) 456-TEA</td>
<td>TEADVOQWL</td>
<td>B*40&lt;sup&gt;f&lt;/sup&gt;</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>NS (9) 457-EAD&lt;sup&gt;d&lt;/sup&gt;</td>
<td>EADVOQQWL</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>NS (9) 460-VQQ</td>
<td>VQQWTWCN</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>NS (9) 613-GLC</td>
<td>GLCPHCINV</td>
<td>A*02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>VP2 (9) 546-TAK</td>
<td>TAKSRYHPL</td>
<td>B*08&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25</td>
<td>0</td>
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<tr>
<td>NS (11) 456-TEA</td>
<td>TEADVOQWL</td>
<td>B*44&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
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<tr>
<td>NS (15) 156-NID</td>
<td>NIDGYIDTCISATFR</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>NS (15) 161-IDT</td>
<td>IDTCISATFRRRGACH</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>NS (15) 336-NLA</td>
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<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
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<td>TTTTVHAKALKERMV</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>NS (15) 431-HAK</td>
<td>HAKALKERMVKLNF</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
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<sup>a</sup> Nomenclature used for peptide naming is as follows: B19 protein abbreviation and then the amino acid length in parentheses, followed by the position of the first amino acid in the protein according to the sequence published by Shade et al. (24). After the hyphen are the first three amino acids in the peptide sequence given.

<sup>b</sup> Sequences in boldface are plausible to contain a shorter epitope due to the overlap of reactive 15-mers.

<sup>c</sup> Percent specific lysis of pulsed autologous target cells in ¹¹⁵Cr-release assays at 50/1. NS(11)456-TEA was defined using ICS and was not tested in the cytotoxic assay.

<sup>d</sup> IFN-γ response in ELISpot relative to PBMC.

<sup>e</sup> Regarded as suboptimal epitopes with one amino acid shift.

<sup>f</sup> NA, not available.
weeks, whereas in all others, they were lost between weeks 15 and 35. From the second week, IgG levels were raised and maintained above 6 IU/ml (23). In S2 and S5, B19 DNA was detected in PBMC throughout the entire follow-up, while this was lost between weeks 13 and 41 in the other individuals. Pool responses were fine mapped to five 15-mers and nine nonamers, of which seven nonamers were considered to represent novel CD8$^+$ T-cell epitopes (Table 2; Fig. 2). By combined methods, it was possible to suggest HLA restrictions for four of these epitopes. When stimulating peptides of different lengths around the sequence of NS(9)456-TEA (see Table 2 for peptide nomenclature) were used in ICS, an additional B*44-restricted 11-mer epitope was detected (data not shown).

Thus, adults presenting with symptomatic B19 infection rapidly develop cellular immune responses with multiple specificities, which rise to high levels and are maintained for many months. The responses do not decay as anticipated but are kept at high levels for a long time, sometimes more than 2 years. This could be the result of continuous antigenic stimulation, analogous to truly persistent low-level CMV infection. Indeed, for three out of five individuals, B19 DNA was detected in peripheral blood for over 6 months. It is possible that B19 persists beyond this time in the bone marrow, in which it is possible that B19 DNA was detected in PBMC throughout the entire follow-up, while this was lost between weeks 13 and 41 in the other individuals. Pool responses were fine mapped to five 15-mers and nine nonamers, of which seven nonamers were considered to represent novel CD8$^+$ T-cell epitopes (Table 2; Fig. 2). By combined methods, it was possible to suggest HLA restrictions for four of these epitopes. When stimulating peptides of different lengths around the sequence of NS(9)456-TEA (see Table 2 for peptide nomenclature) were used in ICS, an additional B*44-restricted 11-mer epitope was detected (data not shown).

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