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Recognition of influenza virus epitope variants by human CTL

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Abstract. Recently, an HLA-B*3501-restricted cytotoxic T-lymphocytes (CTL) epitope in the nucleoprotein ($NP_{418-426}$) of influenza A viruses was identified, which exhibited a high degree of variability. In the present study, the recognition of epitope variants by human cytotoxic T-lymphocytes (CTL) was investigated. Human CD8+ CTL clones were specific for $NP_{418-426}$ epitope variants within one subtype of influenza virus or cross-reactive with hetero-subtypic variants. Positions in the 9-mer were identified as determinants of CTL-specificity. The in vivo existence of T cells cross-reactive with homo- and hetero-subtypic variants of the epitope was confirmed with virus-specific polyclonal T cell populations obtained from HLA-B*3501+ blood donors. Based on CTL recognition patterns and the history of infection as assessed by serology, it was hypothesized that consecutive infections with influenza viruses containing different variants of the epitope select for cross-reactive T cells in vivo.

Keywords: Cytotoxic T-lymphocytes; Epitopes; Escape

1. Introduction

Cytotoxic T-lymphocytes (CTL) play an important role in the control of viral infections. To evade host CTL responses, some viruses accumulate mutations in, or adjacent to, CTL epitopes. Moreover, for influenza viruses, variation in CTL epitopes has been described preventing recognition by specific CTL [1]. Prolonged shedding of mutant virus in a small proportion of individuals may be sufficient to explain the rapid fixation of these epitope mutants [2]. Recently, an HLA-B*3501-restricted CTL epitope was identified

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Table 1
Origin of variants of the $NP_{418-426}$ epitope

Epitope sequence	Epitope name	Period of circulation	Virus subtype	Virus strains used in the present study
LPFDKTTIM	NP_{DTI}	1977–present	H1N1	A/Netherlands/306/00
LPFDKPTIM	NP_{DPI}	1957–1972	H2N2/H3N2	ND
LPFDKSTIM	NP_{DSI}	1972–1980	H3N2	A/Victoria/3/75
LPFEKSTVM	NP_{ESV}	1980–present	H3N2	Resvir-9

(LPFEKSTVM, $NP_{418-426}$), which exhibited a high degree of amino acid variation. All variants emerged in a chronological order and replaced their ancestors. Four main variants were identified (see Table 1), which all retained their anchor residues and bound to HLA-B*3501 molecules [3]. CTL raised against historic variants failed to recognize recent influenza virus strains. We decided to study the recognition of $NP_{418-426}$ variants by specific CTL in more detail using tetramer staining and classical chromium release assays.

2. Materials and methods

Nine different CD8+ CTL clones were obtained as described previously [1]. Polyclonal T cell populations were obtained after stimulation of peripheral blood mononuclear cells (PBMC), with different influenza virus strains as described previously [4]. Influenza viruses A/Netherlands/306/00 (H1N1), A/Victoria/3/75 and Resvir-9 (both H3N2) were used which carry the NP_{DTI}, NP_{DSI} and NP_{ESV} variants of the $NP_{418-426}$ epitope, respectively. The recognition of variants was studied in $^{[51]}$Cr-release assays using virus-infected or peptide-loaded EBV transformed BLCL as target cells. In addition, tetramer binding properties of CTL were studied by flow cytometry. Serum antibodies directed against historic influenza A vaccine strains were measured in a hemagglutination inhibition assay to assess the history of infection of blood donors.

3. Results

Four patterns of reactivity of $NP_{418-426}$-specific CTL clones were observed. Five of the T cell clones were mono-specific for the NP_{ESV} variant of the epitope (reaction pattern 1, Table 2). Two T cell clones reacted with the NP_{DSI} and the NP_{DTI} variant of the epitope (reaction pattern 2). One T cell clone raised against NP_{DSI} cross-reacted with the NP_{DTI}

Table 2
Cross-reactivity patterns of CD8+ T cell clones specific for $NP_{418-426}$

Reaction pattern	ESV	DTI	DSI	DPI
1 (NP_{ESV})[a]	++++	–	–	–
2 (NP_{DTI})	–	++++	++++	–
3 (NP_{DTI})	++++	++++	++++	++++
4 (NP_{DSI})	–	++++	++++	++++

Recognition of individual epitopes was measured in $^{[51]}$Cr-release assays using virus-infected or peptide-loaded target cells and by tetramer staining; – indicates no recognition; ++++ indicates good recognition.

[a] In between brackets, the epitope variants are indicated against which the corresponding CTL clones were raised.

Table 3
Cross-reactivity patterns of CD8+ T cells in influenza virus-stimulated PBMC cultures

Reaction pattern	Stimulation with Resvir-9 (NP_{ESV})			
	ESV	ESV/DTI	ESV/DSI	ESV/DPI
1	++++[a]	+	+	–
2	++++	–	++	–
3	++	–	–	–
	Stimulation with A/Victoria/3/75 (NP_{DSI})			
	DSI	DSI/DTI	DSI/ESV	
1	+++	++	+	
2	+++	–	++	
3	+++	+++	–	
	Stimulation with A/Netherlands/306/00 (NP_{DTI})			
	DTI	DTI/ESV	DTI/DSI	DTI/DPI
1	++	+	++	–
2	–	–	–	–
3	++++	–	+++	++

[a] Indicates the relative frequency of CD8+ T cells specific for one or more epitopes.

and the NP_{DPI} (reaction pattern 4). One of the T cell clones was fully cross-reactive and recognized all variants equally well (reaction pattern 3, Table 2).

Three patterns of recognition were observed with polyclonal T cell populations obtained after stimulation of HLA-B*3501-positive PBMC with three different influenza A viruses containing the NP_{ESV}, NP_{DSI} and the NP_{DTI}, respectively (Table 3). Some donors exhibited a response to all of the individual variants. A small proportion of the NP_{ESV}-specific cells, expanded after stimulation with influenza virus Resvir-9, cross-reacted with NP_{DTI} and NP_{DSI} (reaction pattern 1, Table 3). The NP_{DTI}-specific cells also cross-reacted with the NP_{ESV}-specific cells. Other donors did not respond to NP_{DTI} (reaction pattern 2, Table 3) after stimulation with an influenza A H1N1 virus and only responded to stimulation with influenza A viruses of the H3N2 subtype. In these donors, NP_{ESV}/NP_{DSI} cross-reactive cells were observed, which did not react with NP_{DTI}.

The third recognition pattern revealed CTL specific for NP_{ESV} that did not cross-react with any of the other variants and CTL specific for NP_{DTI} that cross-reacted with NP_{DSI}. The observed reactivity of the T cells with the respective epitope variants exhibited a good correlation with the history of infection of these donors as assessed by serology (data not shown).

4. Discussion

In the present study, CTL were identified specific for a single variant or cross-reactive with various homo- or hetero-subtypic variants of the $NP_{418-426}$ epitope. Positions 4 and 5 of the 9-mer were important determinants of T cell specificity, whereas positions 6 and 8 were not. Most of the T cells in virus-stimulated PBMC cultures were mono-specific for certain variants of the $NP_{418-426}$ epitope. However, a small proportion of the CD8+ cells

showed some degree of cross-reactivity. Some of the CTL specific for NP_{ESV} cross-reacted with NP_{DTI} (reaction pattern 1, Table 3), while other NP_{ESV} specific cells cross-reacted with NP_{DSI} (reaction pattern 2, Table 3). The NP_{DSI}- and NP_{DTI}-specific CTL exhibited a high degree of cross-reactivity in some donors (reaction pattern 3, Table 3). Because the observed cross-reactivity of the CTL populations correlated with the history of infections as assessed by serology, we hypothesized that consecutive infection with viruses containing different variants of the $NP_{418-426}$ epitope may expand a small proportion of CTL which cross-react with these epitope variants as has also been described in a mouse model for influenza [5].

References

[1] J.T.M. Voeten, et al., Antigenic drift in the influenza A virus (H3N2) nucleoprotein and escape from recognition by cytotoxic T lymphocytes, J. Virol. 74 (2000) 6800–6807.
[2] J. Gog, et al., Population dynamics of rapid fixation in CTL escape mutants in influenza A, Proc. Natl. Acad. Sci . U. S. A. 100 (19) (2003) 11143–11147.
[3] A.C.M. Boon, et al., Sequence variation in a newly identified HLA-B35 restricted epitope in the influenza A virus nucleoprotein associated with escape from cytotoxic T lymphocytes, J. Virol. 76 (5) (2002) 2567–2572.
[4] A.C.M. Boon, et al., The magnitude and specificity of influenza A virus-specific cytotoxic T-lymphocyte response in humans is related to HLA-A and B phenotype, J. Virol. 76 (2) (2002) 582–590.
[5] J.B. Haanen, et al., Selective expansion of cross-reactive CD8(+) memory T cells by viral variants, J. Exp. Med. 190 (1999) 1319–1328.