Rapid visual assay of cytotoxic T-cell specificity utilizing synthetic peptide induced T-cell–T-cell killing

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SUMMARY

Synthetic peptides are widely used to define the specificity of $CD8^+$ cytotoxic T-lymphocyte (CTL) clones. When many peptides need to be tested by the standard chromium release assay large numbers of a CTL clone are required. Specific synthetic peptide epitopes induce CTL clones to kill each other. This phenomenon can be directly visualized using an inverted microscope and forms the basis for a convenient assay, which can be performed with as few as 100 CTL per peptide and does not require radiolabelled targets.

The killing of T cells by other T cells has been described in a variety of systems.[1-5] The addition of synthetic peptide epitopes to $CD8^+$ cytotoxic T lymphocyte (CTL), specific for that epitope, will cause those CTL to kill each other[2,3] by inducing apoptosis.[1] This ultimately results in CTL destruction, which can easily be observed with an inverted microscope. Utilizing this phenomenon a simple assay was developed for defining the peptide epitope specificity of CTL clones using synthetic peptides. This visual assay can be performed using 100 to 10^4 CTL for each peptide (as opposed to $1-5 \times 10^4$ for a standard chromium release assay), can be conducted overnight and does not require labelled target cells.

To illustrate the application of the assay for defining CTL epitopes, three previously characterized CTL clones were tested in both this visual and the standard chromium release assay. CTL clones LC13, DM3 and JS18 are specific for the synthetic peptide epitopes FLRGRAYGL, EENLLDFVRF and QLSDTPLIPLTIFVG respectively.[6-8] Two hundred cells of each CTL clone were added per well to a 96-well U-bottom plate in T-cell growth medium containing MLA 144 cell line supernatant and interleukin-2 (IL-2).[7] Synthetic peptides were then added at 100 μM and the plates spun at 100 ***g*** for 5 min and incubated at 37°. After 5 hr the CTL incubated with specific peptide epitope showed morphological changes characteristic of cells undergoing apoptosis.[1] On the following day destruction of nearly all such CTL was clearly visible (Fig 1, Table 1). Addition of an inappropriate peptide had no effect (Fig. 2, Table 1). The CTL clones were also tested for peptide specificity in a standard chromium release assay using 10^4 peptide-coated chromium labelled autologous phytohaemaglutin (PHA) blasts (as target cells) and 5×10^4 CTL per well.[1] All clones, which showed peptide induced CTL–CTL killing in the visual assay, invariably killed autologous target cells coated with the same peptide in the standard chromium release assays. In addition, peptides, which did not induce CTL–CTL killing, did not sensitize autologous target cells for lysis by that clone (Table 1).

We have tested several hundred clones and 171 peptides using this method and have isolated 55 Epstein–Barr virus specific CTL clones specific for five different peptide epitopes (Table 1; S. R. Burrows, A. Suhrbier, R. Khanna and D. J. Moss, unpublished results). We have not found any clone which

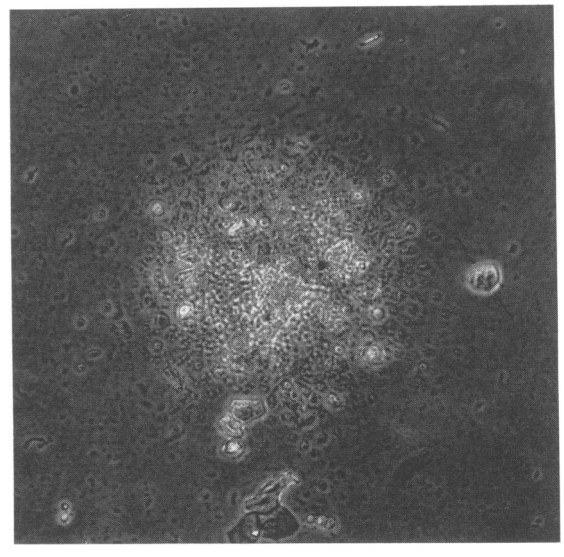

Figure 1. Destruction of LC13 in the presence of synthetic peptide FLRGRAYGL (100 μM). Two hundred cells of CTL clone LC13 after a 16-hr incubation at 37° observed under an inverted phase microscope. Original magnification × 350.

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Table 1. Comparison of the standard chromium release assay and the visual assay. FLRGRAYGL, EENLLDFVRF and QLSDTPLIPLTIFVG are synthetic peptides in single letter amino acid notation. Chromium release values were obtained for peptide-coated autologous PHA blasts in a standard 5-hr assay

	^{51}Cr release assay (% lysis)			Visual assay*		
CTL clone	FLRGRAYGL (%)	EENLLDFVRF (%)	QLSDTPLIPLTIFVG (%)	FLRGRAYGL	EENLLDFVRF	QLSDTPLIPLTIFVG
LC13	40	0	0	+	−	−
DM3	0	58	0	−	+	−
JS18	0	0	36	−	−	+

* Visual assay: + refers to an image similar to that seen in Fig. 1 and − to that seen in Fig. 2.

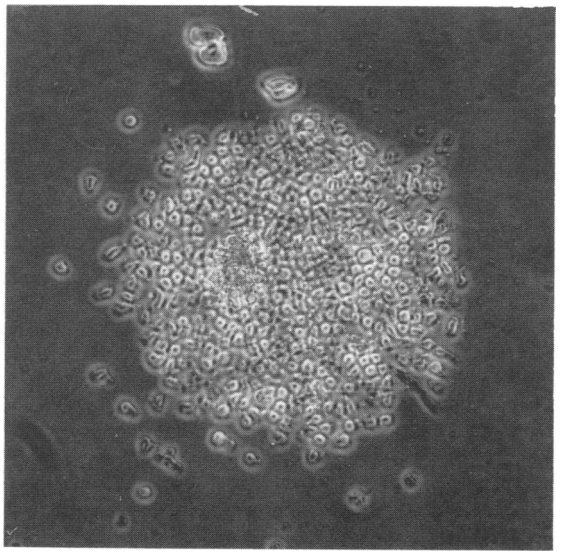

Figure 2. CTL clone LC13 plus synthetic peptide EENLLDFVRF (100 μM).

killed a peptide-coated target cell and was not susceptible to peptide-induced CTL–CTL killing with the same peptide (data not shown). Approximately one in 50 peptides exhibited non-specific toxicity. Such peptides lysed CTL clones known *not* to be specific for the peptide. Toxicity controls were therefore performed for any new peptide using a CTL clone of known specificity (data not shown).

Although some CTL clones only gave low levels of lysis of peptide-coated PHA blasts (e.g. 36%, Table 1) as measured by a 5-hr chromium release assay, after overnight incubation, visual interpretation is conclusive since nearly all CTL are destroyed.

The assay has wide application for screening CTL clones for peptide epitope specificity especially when CTL numbers are limited (e.g. during the early stages of clone expansion).

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