JCI The Journal of Clinical Investigation

The arrival of HLA class II tetramers

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J Clin Invest. 1999;104(12):1669-1670. https://doi.org/10.1172/JCI8943.

Commentary

The innovation of histocompatibility locus antigen (HLA) class I tetramers has revolutionized our understanding of virus-specific T cells (1). To generate this class of reagent, HLA class I molecules are made in Escherichia coli and then refolded with epitope peptides. A peptide tag added at the carboxy terminus of the heavy chain allows the complex to be biotinylated enzymatically, so that 4 HLA molecules bind 1 fluorochrome-marked streptavidin molecule. Although monomeric HLA binding is of too low an affinity to be useful in T-cell recognition, these labeled tetrameric complexes bind stably to antigen-specific CD8+ T cells, because of the avidity they gain by forming tetramers. Tetramer–T cell receptor (TCR) complexes are internalized after binding, which also preserves the staining of the target CD8+ T cells (2). One surprise to come out of this work concerns the deduced prevalence of virus-specific T cells. More than 1% of blood CD8+ T cells stain for single epitopes from persistently infecting viruses such as Epstein-Barr virus (3), human T-cell lymphotropic virus type I (4, 5), or HIV (6). These numbers are more than 10 times greater than those calculated in limiting dilution assays (LDAs), yet there is no evidence that use of tetramers leads to an overestimate of the number of antigen-specific T cells. In acute viral infections, the number of specific CD8+ T […]

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The innovation of histocompatibility locus antigen (HLA) class I tetramers has revolutionized our understanding of virus-specific T cells (1). To generate this class of reagent, HLA class I molecules are made in *Escherichia coli* and then refolded with epitope peptides. A peptide tag added at the carboxy terminus of the heavy chain allows the complex to be biotinylated enzymatically, so that 4 HLA molecules bind 1 fluorochrome-marked streptavidin molecule. Although monomeric HLA binding is of too low an affinity to be useful in T-cell recognition, these labeled tetrameric complexes bind stably to antigen-specific CD8$^+$ T cells, because of the avidity they gain by forming tetramers. Tetramer–T cell receptor (TCR) complexes are internalized after binding, which also preserves the staining of the target CD8$^+$ T cells (2).

One surprise to come out of this work concerns the deduced prevalence of virus-specific T cells. More than 1% of blood CD8$^+$ T cells stain for single epitopes from persistently infecting viruses such as Epstein-Barr virus (3), human T-cell lymphotropic virus type I (4, 5), or HIV (6). These numbers are more than 10 times greater than those calculated in limiting dilution assays (LDAs), yet there is no evidence that use of tetramers leads to an overestimate of the number of antigen-specific T cells.

In acute viral infections, the number of specific CD8$^+$ T cells is even greater. In acute infectious mononucleosis, up to 44% of blood CD8$^+$ T cells can react with a single Epstein-Barr virus epitope (3). Similar numbers are found in mice infected with lymphocytic choriomeningitis virus (LCMV) (7), influenza virus (8), and *Listeria monocytogenes* (9). These huge expansions are short-lived — most of the T cells die by apoptosis, which may explain the discrepancy between the direct counts of specific T-cell subpopulations and the values measured by the LDA. In the LDA, single clones have to survive, divide, and differentiate before they can be detected, so this assay may measure long-term memory T cells, whereas tetramers stain both memory cells and an expanded effector population that can no longer divide (10).

It has been questioned whether all of the CD8$^+$ T cells that proliferate in response to acute infections actually function in immune responses (11). Here too, HLA class I tetramers have provided useful information. Once T cells expressing specific receptors have been stained using these tetramers, their function can be assessed by sorting stained cells or by costaining with antibodies to cytokines, chemokines, or perforin. Despite clues that in most instances such cells are active, there are reports of impaired function. Mice infected with high-titer, aggressive LCMV have expansions of tetramer-staining cells that seem functionless (12). In addition, dysfunctional tumor-specific T cells were found in a patient with melanoma (13), and LCMV-infected mice that had been depleted of CD4$^+$ T cells expressed CD8$^+$ T cells that stained with an appropriate tetramer but appeared functionless (11). In HIV infection, CD4$^+$ T-cell function is impaired early, raising the question of whether all of the CD8$^+$ T cells seen in HIV-infected individuals are active against the virus. There is evidence that these cells function, but the issue remains of how CD4$^+$ T cells influence the CD8$^+$ T-cell response.

This uncertainty demonstrates the need to develop HLA class II tetramers that could be used to explore the role of CD4$^+$ T cells in infections, autoimmunity, and atopic diseases. Several groups have tried to develop such reagents, but all have met with technical difficulties. Soluble α and β chains from H-2 IE^k have been made in *E. coli*, and then refolded in the presence of excess peptide and biotinylated (14). Monomeric, dimeric, trimeric, and tetrameric complexes formed could be used to study T-cell activation, but yields were low and the method has been difficult to reproduce with other class II alleles. Successful class II tetramer formation requires interaction of 3 components — α and β chains and the peptide — making it more complex than the corresponding process for class I molecules, which can occur in 2 steps. We have attempted to refold HLA DR1 made by *E. coli* without success, despite having added leucine zippers to link the peptide to the β chain (A. Kelleher, unpublished results). However, *E. coli* expression has been used successfully by another group (L. Stern, personal communication).

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Crawford et al. (15) developed a second approach that uses recombinant baculovirus or direct transfection to express class II molecules in insect cells, taking advantage of the ability of these cells to express HLA class II molecules at high levels in the absence of antigen processing. These authors linked a number of peptide epitopes to the amino terminus of the β chain using a short flexible linker to produce H-2 IE^k and H-2 IA^k, from which they prepared biotinylated tetramers. These tetramers stained T-cell hybridomas; the intensity of their staining correlat-

ed with their affinity for the TCR and the level of TCR expression. Antigen-specific T cells were detectable in TCR transgenic mice. HLA DR4 tetramers were also made (J. Kappler, personal communication). We have prepared HLA DR1 coupled to an HIV gag peptide in this way (A. Kelleher, unpublished results). A drawback to this approach is that the peptide has to be engineered into the construct, which is cumbersome, but in this issue of the *JCI*, Novak et al. (16) describe production of HLA DR4 tetramers that contain an influenza virus epitope. DR4 was expressed in empty form in insect cells, with the 2 chains linked at their carboxy termini by a leucine zipper. Empty DR4 was purified and then soaked in the required epitope peptide to give a stable molecule that could be biotinylated and used to stain influenza virus–specific $CD4^+$ T cells.

This staining confirms the specificity of the reagent and affords Novak and colleagues the first glimpse of a human antiviral $CD4^+$ T-cell response. They could detect no staining of T cells in blood from immune humans, but stimulation in vitro with influenza antigen induced $CD4^+$ T cells that stained with the tetramer. By costaining these cultures with 5-carboxyfluorescein diacetate succinimidyl ester, which dilutes exponentially as the cells divide, Novak et al. could calculate the number of divisions that the cells completed — up to 10 — within 7 days of antigen stimulation. These data allow the number of precursors in the original blood sample to be determined, avoiding the considerable difficulties associated with the LDA. Precursor frequency determined by these calculations agrees with estimates from the LDA (< 1 in 10^4).

The number of virus-specific $CD4^+$ T cells appears to be lower than that of the $CD8^+$ T cells cited above, but the latter were measured during persisting infections. Influenza virus is eliminated after each attack; specific $CD8^+$ T cells can be found in the blood of healthy adults but at similar low levels (1 in 10^4). It remains to be seen whether the $CD4^+$ T-cell response to a persisting or acute virus is smaller than the massive $CD8^+$ T-cell response. Many questions about the human $CD4^+$ T cell response to natural antigens can now be explored in detail.

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