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Antibodies and Lentiviruses That Specifically Recognize a T Cell Epitope Derived from HIV-1 Nef Protein and Presented by HLA-C

Alon Herschhorn,*1 Wayne A. Marasco,† and Amnon Hizi*

HIV selectively downregulates HLA-A and -B from the surfaces of infected cells to avoid detection by the immune system. In contrast, the HLA-C molecules are highly resistant to this downregulation. High expression level of HLA-C on the cell surface, which correlates with a single nucleotide polymorphism, is also associated with lower viral loads and slower progression to AIDS. These findings strongly suggest that HIV-1–derived peptides are efficiently presented by HLA-C and trigger the elimination of infected cells. Accordingly, the ability to detect these HLA-C–peptide complexes may be used for therapeutic targeting of HIV-1–infected cells and for measuring effective presentation of vaccine candidates after immunization with HIV-1–related proteins or genes. However, low level of HLA-C expression on the cell surface has impeded the development of such complex-recognizing reagents. In this study, we describe the development of a high-affinity human Ab that specifically interacts, at low pM concentrations, with a conserved viral T cell epitope derived from HIV-1 Nef protein and presented by HLA-C. The human Ab selectively detects this complex on different cells and does not interact with a control complex that differed only in the presented peptide. Engineering lentiviruses to display this Ab endowed them with the same specificity as the Ab, whereas coexpressing the Ab and Fas ligand enables the lentiviruses to kill specifically Nef-presenting cells. Abs and pseudoviruses with such specificity are likely to be highly valuable as building blocks for specific targeting and killing of HIV-1–infected cells.

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During the intracellular replication of HIV type 1, some viral proteins are cleaved into peptides in the cell cytoplasm. These peptides are actively transported into the endoplasmic reticulum, where they are bound by HLA class I molecules. The assembled HLA–peptide complexes are subsequently transported into the plasma membrane of the infected cell and can selectively mark the infected cells for an immune attack by specific CTLs (1, 2). Cleaved peptides derived from HIV-1 proteins can potentially be presented by either HLA-A, HLA-B, or HLA-C, which are expressed on virtually every nucleated cell in the human body (3). Presentation on the cell surface specifically labels HIV-1–infected cells and, therefore, can be further used for a selective detection and targeting of these cells. A similar approach was successfully used to analyze the expression level of HLA-A2, presenting a viral peptide derived from human T cell leukemia virus type 1 (4), or to direct a toxin-conjugated Ab to HLA-A2–expressing cancer cells (5). However, applying this strategy to HIV-1–infected cells should take into account the ability of HIV-1 to interact with HLA complexes on the surfaces of HIV-infected cells (1, 2). HIV-1 evades the immune response by selectively downregulating HLA-A and HLA-B molecules from the infected cell surface (6, 7). Downregulation is very extensive (8), although not absolute, as evident from the selective pressure of HLA-B–restricted CTLs on HIV-1 adaptation (9, 10). In contrast, HIV-1 is unable to remove the HLA-C molecules from the cell surface as this will expose the infected cells to an attack by NK cells (6, 7). Accordingly, targeting viral peptides that are presented by HLA-C may be more effective than targeting other HLA–peptide complexes. This notion is further supported by the growing evidence for the key role of HLA-C in the development of the immune response to HIV-1. Despite the low physiological levels of HLA-C expression on the cell surface, relative to HLA-A and HLA-B, specific HLA-C–restricted CTLs may be as effective as the ones generated against other HLA–peptide complexes. Such responses can account for as much as 54% of the total CTL response in individuals, and these CTLs are functionally and phenotypically identical to the other HLA-restricted CTLs (11). In another study, 17% of the peptides that were associated with specific CTL responses were HLA-C restricted, whereas 22% were HLA-A restricted and 60% HLA-B restricted (10). A single HLA-C allele (HLA-C*07:01) was also found to be the most frequent in presenting peptides derived from HIV-1 Nef protein (subtype A) (12). Moreover, a host genetic variant, located upstream of the HLA-C gene (−35C), correlated with increased expression of HLA-C and was associated with lower viremia and slower progression toward AIDS in recent studies (13, 14). Nevertheless, in some individuals that carry the protective variant, HIV-1 may still counteract this protection by enhancing Nef-mediated viral infectivity and by manipulating both T cell functions and Ag presentation (15). Notably, even when HIV-1 overcame this protection, there was no evidence for an increased...
All complexes contained three components: HLA H chain, β2-microglobulin (β2m), and a peptide derived from either a specific HIV-1 protein or an unrelated cellular protein. They were prepared according to National Institute of Allergy and Infectious Diseases Tetramer Core Facility protocols (http://research.yerkes.emory.edu/tetramer_core/) with several modifications described later. The plasmids that carry the genes for HLA-C*07:02 H chain and β2m were synthesized by Genemed Synthesis (San Antonio, TX), and Nef2 by GL Biochem (Shanghai, China).

Preparation of HLA–peptide complexes

All complexes contained three components: HLA H chain, β2-microglobulin (β2m), and a peptide derived from either a specific HIV-1 protein or an unrelated cellular protein. They were prepared according to National Institute of Allergy and Infectious Diseases Tetramer Core Facility protocols (http://research.yerkes.emory.edu/tetramer_core/) with several modifications described later. The plasmids that carry the genes for HLA-C*07:02 H chain and β2m were synthesized by Genemed Synthesis (San Antonio, TX), and Nef2 by GL Biochem (Shanghai, China).
envelope protein able to infect a wide variety of cells. The viruses were allowed to infect 721,221 cells at high multiplicity of infection in the presence of 5.6 μg/ml polybrene. After 72 h, the cells were further sorted twice for high level of C*07:02 expression after labeling with W6/32 mAb and FITC-conjugated secondary Ab, using FACSAria (BD Biosciences, San Jose, CA). Sorted cells were maintained in a medium containing 4 μg/ml puromycin to avoid genetic reversions. PV-C3 was prepared by co-transfecting 293 T cells with 10 μg pCMVΔ8.2Gp120, 7.5 μg pLeGO-IgG (35), and then μg pCDNA3.1-ScFvFc-CD28-gp41. Control PVs were prepared similarly, but with pCDNA3.1-PS1-ScFvFc-CD28-gp41, encoding an unrelated control Ab instead of the C3 Ab. PV-C3-Fasl was prepared by the C3 but with 4 μg pigIg2-Fasl plasmid replacing the pLeGO-IgG plasmid. In this case, apoptosis of some 293T cells was observed during preparation, but the cells could still support PV production as determined by binding of the PV to Nef1-expressing cells and by RT activity assay, which measured the titer of the generated PV.

Flow cytometry

About 10^6 sorted cells were washed twice with RPMI 1640 without serum and divided into two sets: one incubated with 100 μM Nef1 peptide and the other with 100 μM control peptide (Conopep). ScFv binding was tested by incubating with the cells for 30–60 min, washing the cells, and incubating with 1 μl mouse IgG anti-C-mycc mAb (as C-mycc is incorporated at the C terminus of all ScFvs). Cells were washed, incubated with fluorescein-conjugated goat anti-mouse IgG mAb, washed, washed again, and analyzed with FACSCount (BD Biosciences). Binding of C3-IgG and PV particles were tested by incubating them with each set of cells (pre-incubated with either Nef1 or control peptide) for 40 min. The cells were washed, incubated with 1 μl PE-conjugated donkey anti-human IgG or 1.2 μl DyLight649-conjugated donkey anti-human IgG Abs for 15 min, and then further washed. The cells were analyzed with FACSCount (BD Biosciences). All secondary Abs were Fab2 fragments from Jackson ImmunoResearch Laboratories (West Grove, PA). Washes were performed with PBS containing 5% FCS, and incubations were done on ice.

Confocal microscope

Binding was tested essentially as described for the flow cytometry, but only DyLight649-conjugated secondary Abs were used to label the C3-IgG or PV-C3. The last step was followed by fixation with 1% paraformaldehyde, and the cells were cordo-spun for 5 min onto a microscope slide, mounted with a drop of antifade solution, and covered with a coverslip. Images were acquired with a Zeiss LSM 510 confocal microscope (Carl Zeiss, Germany).

**Results**

Screening phage display libraries

To isolate specific Abs against the recombinant HLA-C*07:02 presenting the Nef1 peptide (designated HLA-C*07:02–Nef1) (Table I), we assembled a biotin-tagged complex. For this purpose, βm protein was refolded and chemically tagged with biotin. The tagged βm protein was then incubated with a denatured, recombinant HLA-C*07:02 H chain and the Nef1 peptide to allow complex formation. The resulting complex was purified, and the presence of correctly folded HLA-C*07:02–Nef1 was confirmed by both ELISA and dot blot assays using the W6/32 Ab (Supplemental Fig. 1). The biotinylated complex was next used for selection of three phage libraries. These libraries display human ScFv Abs on the surfaces of the phages. The first two libraries, designated Metha1 and Metha2, were constructed from 2.75 × 10^8 B cells that were isolated from the peripheral blood of 57 nonimmunized human volunteers. The Metha1 library was constructed through the random assembly of 2.75 × 10^5 V_H genes with 1.6 × 10^6 V_κ and 1.6 × 10^5 V_λ genes and yielded 1.2 × 10^10 member library. The Metha2 library contained random combinations of the V_κ genes with only the V_λ genes and consisted of 1.5 × 10^10 members (36). The third phage library, Griffin1, was a semisynthetic library of 1.2 × 10^9 different clones and was based on the lox library (37). This library contained the vast majority of V gene segments used in vivo as building blocks, and these were further randomly mutated at the CDR3 (CDR3) loop to increase the diversity of the library.

Selection of the three phage libraries was directed to isolate Abs against specific epitopes of the presented Nef1 peptide and possibly nearby HLA-C*07:02 residues. To select for such a specificity toward the Nef1-presenting complex, we applied a subtraction step (5) by incubating the phages with two different complexes: biotin–HLA-C*07:02–Nef1 and an excess of control, nonbiotinylated HLA-C*07:02–gp120. The excess nonbiotinylated HLA-C*07:02–gp120 was used as a trap for binders against epitopes common to all HLA-C*07:02 complexes, and streptavidin-coated magnetic beads were applied to retrieve the phages bound to biotin–HLA-C*07:02–Nef1. After four rounds of panning against each library, the phage populations were tested by ELISA with the two complexes. Phages selected from both Metha1 and Metha2 libraries showed enrichment of binders specific to the biotin–HLA-C*07:02–Nef1 complex and a much lower binding to the control complex (Fig. 1). In contrast, phages selected from Griffin1 library showed comparable binding to both complexes and probably contained only binders to common epitopes on the HLA-C*07:02 molecule (Fig. 1). Based on this analysis, the phage populations from the Metha libraries were then screened to identify monoclonal ScFv Abs expressed from single clones. This resulted in identifying four unique clones that were isolated for further evaluations: C3, A4, D6, and E9.

**Soluble ScFvs**

To test the fine specificity of the soluble ScFvs, they were tested against three different biotinylated complexes: the biotinylated HLA (B-HLA)-C*07:02–Nef1 and two control complexes. The first control complex was B–HLA-C*07:02–Conopep, which differs from the previous complex by presenting an unrelated peptide (Conopep). Conopep is derived from human cyclin-dependent kinase interacting protein and was found as a natural C*07:02 ligand of human 721,221 cells (25). Therefore, Conopep serves as an ideal control for an endogenous peptide that is naturally processed and presented by HLA-C*07:02 on human cells (hence, Abs interacting with this complex may bind normal cells without any specificity toward HIV-infected cells). The second control complex used was B–HLA–B*08:01–Nef2, which presented a second unrelated peptide derived from HIV-1 Nef on a different recombinant HLA molecule (HLA-B*08:01). When the identified Abs were tested against the different HLA complexes, C3 and E9 ScFvs showed

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**Table I. Identity of the residues in the sequence of Nef1 peptide and their prevalence in 661 HIV-1 isolates**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>K</th>
<th>R</th>
<th>Q</th>
<th>D (E/F)*</th>
<th>I</th>
<th>L</th>
<th>D</th>
<th>L</th>
<th>W</th>
<th>V</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity (%)</td>
<td>73.7</td>
<td>99.1</td>
<td>94.1</td>
<td>62.9 (99.1)</td>
<td>98.9</td>
<td>99.4</td>
<td>98.8</td>
<td>98.5</td>
<td>97.9</td>
<td>84.4</td>
<td>97.1</td>
</tr>
</tbody>
</table>

The sequences that correspond with this T cell epitope were retrieved for all sequences available in the Los Alamos HIV database (http://www.hiv.lanl.gov/) and exported to an Excel (Microsoft, Redmond, WA) worksheet. The frequency of each residue at each position was then calculated.

*At this position, 62.9% of the sequences contained an Asp residue, whereas 99.1% of the sequences contained either an Asp or a Glu residue at this position.*
comparable binding efficiencies to B-HLA-C*07:02–Nef1. Nota-

FIGURE 1. Polyclonal phage ELISA using the phage populations that was per-
formed after four panning cycles. Selection of Metha1, Metha2, and Griffin1 phage dis-
play libraries against biotinylated Ag was performed as previously described (5, 28),
but washes in all panning cycles were done 10–12 times with PBS containing 2% skim
dry milk and 0.1% Tween 20 and then twice with PBS. From the second round of se-
lection, about a 2-fold excess of unrelated and nonlabeled HLA control complex (C*07:02–gp120) was incubated, along with the C*07:02–Nef1 complex, during the binding step of the phages (5). This was done to specifically enrich for phages specific against epitopes formed by the Nef1 peptide and HLA protein interface. Bulk phage popu-
lations were tested for binding to the biotin-
HLA-C*07:02–Nef1 complex as well to the control complex (biotin–HLA-C*07:02–gp120) and to NeutrAvidin.

The four ScFvs were next tested for their ability to bind cells presenting the HLA-C*07:02–Nef1 complex. Binding was assessed with 721.221 human B lymphocytes that overexpress the HLA-C*07:02 complex on their surfaces after a lentivirus-mediated gene transfer of the HLA-C*07:02 allele. These cells, designated 721-221, exclusively express the HLA-C*07:02 allele, as non-infected 721.221 lymphocytes are characterized by a complete ab-
sence of HLA-A, -B, and -C expression on their surfaces (38). The expression of HLA-C*07:02 on these cells was further verified with the W6/32 Ab (Fig. 3A). Of the four tested ScFvs, C3 showed the strongest and most specific binding to 721-C*07:02 lympho-
cytes that were preincubated with Nef1 peptide (Fig. 3C); hence, it was studied further. In flow cytometry, C3-ScFv bound specifically 721.221 lymphocytes that presented the HLA-C*07:02–Nef1 complex in a dose-dependent manner over a wide concentration range (Fig. 4A). Specific binding could be detected from as low as ~5 nM ScFv, whereas a minor nonspecific binding to lymphocytes that were preincubated with Conpep was observed at only high concen-
trations (Fig. 4A, 4C). The binding of W6/32 to cells presenting each peptide confirmed that both complexes were presented at comparable levels on both sets of cells (Fig. 4E). Because addition of exogenous peptides was reported to increase cell surface ex-
pression of the HLA class I molecules (39), the extent of binding of W6/32 was also measured for each set of cells at a very low con-
centration of each peptide (table in Fig. 4E). When the signal from high peptide concentration was compared with the signal from the low peptide concentration, a shift of ~25 mean fluorescence in-
tensity units was observed for both the Conpep and Nef1 peptides, confirming that these two peptides were actually bound and effec-

FIGURE 2. Binding of monoclonal ScFv Abs to recombinant HLA complexes, as detected by ELISA. A. Bacterial-periplasmic fractions, which contain the specific soluble ScFvs, were tested against NeutrAvidin or three different biotinylated HLA complexes that were previously immobilized through NeutrAvidin, as specified. Although the initial panning was done with the HLA-C*07:02 complex, containing the gp120-derived peptide as control, further experiments showed that the complex did not fold very efficiently and, therefore, the gp120-derived peptide was replaced in further experiments by Conpep. B. All immobilized HLA complexes were also detected with the W6/32 Ab to assess their amounts in the reactions.
tively presented by the HLA-C*07:02 molecule on these cells. Notably, incubation of Nef1-presenting lymphocytes with 505 nM C3-ScFv has resulted in a similar binding pattern as that of W6/32, suggesting that C3-ScFv bound all available molecules on the cells (Fig. 4A, 4E). Binding of C3-ScFv to Nef1-presenting lymphocytes correlated also with the density of the Nef1 peptide on these cells (Fig. 4B, 4D), demonstrating once again that the binding is specific to this complex of HLA-C*07:02 with the Nef1 peptide. In all cases, there was no significant cross-reactivity with Conpep-presenting lymphocytes (and only very minor binding could be detected at high peptide concentrations).

**C3-IgG Ab**

C3-ScFv was further tested for its ability to identify the Nef1 peptide presented on Jurkat cells, which are T lymphocytes that carry the human HLA-C*07:02 allele. These cells constitutively express on their surfaces low levels of this complex, as opposed to the over-expression of HLA-C*07:02 by the 721.221 cells. Unexpectedly, binding to Nef1-presenting Jurkat cells could not be detected by C3-ScFv, and this was probably due to the low levels of the HLA-C*07:02 complex on these cells (data not shown). In an attempt to improve its binding ability, C3-ScFv was converted into a full-length human IgG molecule, which retains two identical Ag binding sites for its target. For efficient expression, the 2A self-processing peptide was used to facilitate equimolar expression of the L and H chains (see *Materials and Methods* and Ref. 31), and this resulted in high expression level of the C3-IgG in 293T cells. C3-IgG molecule showed an exceptionally strong binding and could easily detect C*07:02–Nef1 on different types of cells. Nef1-presenting B lymphocytes, which overexpress the HLA-C*07:02 allele, could be detected at as low as 10 pM C3-IgG, whereas Jurkat T cells, which express lower levels of endogenous HLA-C*07:02, were detected at a slightly higher concentration of ~66 pM (Fig. 5B, 5C). C3-IgG could also bind Lck cells, which are human B lymphocytes expressing the HLA-C*07:02 from an endogenous gene, after they were pulsed with Nef1 peptide (data not shown). The binding efficiency of C3-IgG was dose-dependent in all instances (Fig. 5B, 5C), and no significant binding was detected to B lymphocytes that express a different HLA (HLA-B8) and were pulsed with Nef1 peptide (Fig. 5A). To validate the specificity of C3-IgG, its binding to Nef1-presenting cells (either 721-C*07:02 or Jurkat) was tested in the presence of recombinant soluble HLA-C*07:02 complexes (Fig. 5D). C3-IgG binding to the cells was drastically reduced when the soluble HLA-C*07:02–Nef1 complex was added to the reaction as a competitor, thus confirming the binding specificity. As expected, the presence of the control HLA-C*07:02–Conpep did not significantly affect the extent of binding. Specific binding of C3-IgG was also detected by confocal microscopy (Fig. 5E, 5F). In this method, single Nef1-presenting lymphocytes (either 721-C*07:02 or Jurkat cells) displayed a staining ring that is typical of membrane binding of C3-IgG, whereas lymphocytes pulsed with the Conpep showed merely a background signal.

**Lentiviruses expressing the C3 Ab**

To explore further the ability to detect Nef1-presenting cells, we engineered HIV-1–based lentiviruses that were pseudotyped with the C3 Ab (designated PV-C3). Displaying this Ab on the virus surface was facilitated by fusing C3 to the trans membrane portion of CD28 and incorporating the putative HIV-1 packaging signal at the C terminus of the fused protein (32) (see *Materials and Methods*). In this format, multiple C3 molecules are displayed on the viral particle, enabling simultaneous interactions with more than one cellular HLA-C*07:02–Nef1 complex and increasing the total avidity of the displayed C3 molecules. When the binding...
ability of the lentiviral particles was tested, PV-C3 specifically detected the recombinant HLA-C*07:02–Nef1 complex, with no significant binding to the HLA-C*07:02–Conpep control complex (Fig. 6A) (40). In contrast, a control PV that displayed an unrelated Ab [specific to cyclin T1 (32)] did not react with both Nef1 and control peptide-presenting complexes. In flow cytometry, PV-C3 showed a similar pattern of binding to the Nef1-presenting Jurkat cells as that of the parental C3-IgG, with a wide range of binding and high specificity (Fig. 6B). Minor binding to Conpep-presenting lymphocytes could be detected only at high peptide concentrations. The specific binding of PV-C3 to the HLA-C*07:02–Nef1 complex was further supported by detecting simultaneously the binding of PV-C3 and W6/32 to Nef1-presenting B lymphocytes in two different assays (Fig. 6C, 6D). Confocal microscopy has demonstrated that both PV-C3 and W6/32 Ab were colocalized to the cell membrane surface with some low intracellular localization of W6/32 that was probably due to a minor internalization (Fig. 6C). Flow cytometric analysis further showed a direct correlation between the extent of PV-C3 binding and the binding of W6/32 to the lymphocytes (Fig. 6D). In both analyses, simultaneous incubation of Conpep-presenting lymphocytes with PV-C3 and W6/32 resulted in the binding of only the latter Ab to the cells.

**Lentiviruses coexpressing C3 and Fas ligand**

The ability to pseudotype virions enabled us also to coexpress both Fas ligand (41) and C3 Ab on the surface of the same virion particle. The rationale behind such display was that the Fas ligand may potentially interact with a FasR on the target cells and consequently induce apoptosis of these cells. Therefore, the combined presentation endowed the PV-C3-FasL particle with both a recognizing ability (by the C3 Ab) and a killing capacity (by the Fas ligand) that are vital components for directing specific cell killing activity. When tested, PV-C3-FasL showed similar binding specificity as PV-C3 (data not shown). Interaction of PV-C3-FasL with Nef1-presenting Jurkat cells resulted in a significant and specific (though relatively low) apoptosis of these cells, leading to their elimination. PV-C3-FasL killed between ~6 and 19% of the Nef-presenting cells in a concentration-dependent manner (Fig. 6E). Nevertheless, at high PV-C3 concentrations, the higher killing of Nef-presenting cells was accompanied by some nonspecific killing of Conpep-presenting lymphocytes.
presenting cells (yet, killing of Nef-presenting cells was always higher).

**Discussion**

We have developed reagents that can selectively detect the HLA-C presenting a peptide derived from HIV-1 Nef on the surfaces of human cells. HLA-C*07:02–Nef1 complex represents an optimized target for targeting HIV-1–infected cells. The HLA-C*07:02 allele is frequent in the population, and the Nef1 peptide is highly conserved. Notably, a Nef1 peptide was also reported to bind and be presented by HLA-C*07:01 (18, 23) (Los Alamos HIV database at http://www.hiv.lanl.gov), which differ in only two amino acid residues from HLA-C*07:02. Accordingly, HLA-C*07:01–Nef1 may also be recognized by the C3 Ab, and this

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**FIGURE 5.** Specific binding of C3-IgG to different cells that present the HLA-C*07:02–Nef1 complex. **A**, Flow cytometric analysis of the binding of C3-IgG to three different types of cells preincubated with Nef1 (green), Conpep (red), or secondary Ab (blue). **B**, The flow cytometric binding results of C3-IgG to B lymphocytes stably expressing the HLA-C*07:02 were used to generate a dose-response curve for the binding of C3-IgG. Experimental data represent average fluorescence ± SD for each concentration, n = 3. **C**, Similar to **B**, but the binding was tested with Jurkat cells (T lymphocytes) that express only low, endogenous levels of HLA-C*07:02. Color codes are as in **B**. **D**, Binding of 290 pM C3-IgG to 721-C*07:02–Nef1 cells (left) or 4.2 nM C3-IgG to Jurkat cells presenting Nef1 (right) in the absence or presence of similar amounts of correctly refolded (determined by ELISA with W6/32 Ab), different B-HLA-C*07:02 complexes. **E** and **F**, Detection of C3-IgG binding to HLA-C*07:02–Nef1 presenting cells by confocal microscopy (original magnification ×63; digital scan zoom ×2). Binding of 4.4 nM C3-IgG to B lymphocytes (**E**) or Jurkat cells (**F**), which present either Nef1 or control peptide by HLA-C*07:02, was visualized by a confocal microscope after detection with DyLight649-conjugated secondary Abs. In each panel, the left side shows the emission observed above 640 nm, and the right side shows the cells under phase contrast setting.
ability has to be tested. Such recognition will expand the use of the C3 Ab, as within the HLA-C group both HLA-C*07:01 and HLA-C*07:02 alleles are dominant in many human populations worldwide. Nef1 peptide was identified based on various reports and after an exhaustive alignment with different HIV-1 isolates. Direct evidence shows that HLA-C*07:02 presenting this peptide explicitly resists any downregulation of the complex by HIV-1 Nef, and cells presenting this complex retain susceptibility to a specific CTL attack (22). Additional reports demonstrated that only a limited number of mutations have been observed in this peptide.

FIGURE 6. Functional presentation of C3 Ab and the Fas ligand on lentiviruses. A, Equal amounts of PV-C3 or PV-Control (0.069 pmol dTTP/30 min of RT activity) were tested for binding recombinant B-HLA-C*07:02–Nef1 or B-HLA-C*07:02–Conpep complexes in ELISA. W6/32 Ab was used to measure to amount of the complexes in the reaction. B, Dose-response curve of flow cytometric analysis for the binding of different amounts of PV-C3 (measured by RT activity, as specified) to Jurkat cells preincubated with Nef1 (green) or with Conpep (red). C, Specific binding of PV-C3 to the HLA-C*07:02–Nef1 presented on B lymphocytes was tested by simultaneously incubating the cells with PV-C3 and the W6/32 Ab. Binding of PV-C3 was detected with DyLight649-conjugated secondary Abs, and binding of W6/32 Ab to HLA-C*07:02 was detected with fluorescein-conjugated secondary Abs. Emission pattern of the two fluorophores was recorded with a confocal microscope (original magnification ×63). D, The cells from A were analyzed by flow cytometry, and the results are shown as a dot plot of the emission of each cell at 661 nm (x-axis, DyLight649) and 530 nm (y-axis, fluorescein). The percentages of cells from the total population are displayed in each quadrant (except the lower left). Measurements are displayed by the Cylogic software (CyFlo, Turku, Finland). E, Left panel, A schematic presentation of the PV-C3-FasL particle. Right panel, The killing effect of PV-C3-FasL on Nef1-presenting Jurkat cells (green) or Conpep-presenting Jurkat cells (red) was tested by incubating the different cells with PV-C3-FasL particles for 16 h at 37°C. Apoptosis was recorded by measuring the loss of mitochondrial membrane potential with the cell-permeable green-fluorescent lipophilic dye DiOC6 as previously described (40).
and PVs with TCR-like specificity are likely to be highly valuable in developing vaccines against HIV-1 infection. For example, these Abs may enable the precise characterization of the infected cells the transport of HLA-C complexes that display epitopes that are formed in the interface of the peptide and nearby residues in the H chain of the HLA protein. To achieve this aim, the successful panning was carried out with a mix of a biotinylated C*07:02–Nef1 complex and a nonbiotinylated HLA-C*07:02 control complex, presenting a gp120-derived peptide (as bait for Abs against undesired epitopes). It is important to note that although this complex was prepared in a similar manner as the HLA-C*07:02–Nef1 (with identical concentrations of all components), later experiments showed that there were some difficulties in refolding it correctly, as a lower yield of refolded complex was observed in these preparations in comparison with that of the HLA-C*07:02–Nef1. Nevertheless, using excess of the control complex still allowed us to isolate four unique ScFv Abs: three of them showed specific binding to the recombinant HLA-C*07–Nef1 complex. The fourth ScFv, A4, reacted most probably with an epitope found exclusively on the HLA-C molecule (as it reacted with similar complex that presented a control peptide), demonstrating that the selection scheme was not absolutely selective for HLA-C–Nef1 complex. Within the three specific ScFvs, C3 showed the strongest binding to cells expressing HLA-C*07:02–Nef1. The inability of the other two Abs to detect the HLA-C*07:02–Nef1 on cells can result from different potential reasons. D6 may have low affinity that does not enable it to detect the cell-expressed complex under the experimental conditions used, whereas E9 may bind an epitope that is either modified or inaccessible during presentation on cells.

Conversion of C3-ScFv to C3-IgG substantially increased the binding capacity of the molecule. The enhanced ability of C3-IgG to detect the HLA-C*07:02–Nef1 on surfaces of cells was demonstrated with several cell lines and by using different techniques. According to the data presented, C3-IgG has a notably high affinity to the HLA-C*07:02–Nef1 as it detects, at picomolar concentrations, low levels of the complex on the surfaces of cells. Our study also shows that complexes of HLA-C presenting different peptides can be readily detected and visualized with high-affinity Abs. To our knowledge, this is the first report on the ability to detect and target specifically HLA-C–presenting complexes by Abs and presenting PVs. These reagents can be further applied to several fields of study. Such binders, used as either Abs or PVs with TCR-like specificity, are likely to be highly valuable in developing vaccines against HIV-1 infection. For example, these Abs may enable one to assess the level of HLA–peptide complexes presented after infection with HIV-1–related proteins or genes. Accordingly, they can be used to select and optimize the candidates for effective vaccinations. The Ab reagents may also be useful in tracking in the infected cells the transport of HLA-C complexes that display viral peptides. This can allow the precise characterization of the dynamics of viral peptide presentation. More importantly, as epitomized by coexpressing C3 and Fas ligand on PVs (Fig. 6E), Abs and PVs with TCR-like specificity are likely to be highly valuable as building blocks for specific targeting and killing of HIV-1–infected cells.

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