specific T cell line after several stimulations *in vitro* (Fig. 2E, first plot) and their peptide specificities determined by individual peptide-loaded DimerX staining (Figure 2E). One to nine percent of $CD8^+$ T cells were specific for each one of the selected HIV-1B peptides (Figure 2F).

Cytotoxic activity of HIV-1 peptide-specific CD8+ T cells derived from HIV-1 naive donors

Next, to test for potential cytolytic activity of the HIV-1 peptide-specific T cell lines, T2 hybridoma cells were pulsed with A0201 restricted HIV-1B peptide pools and specific lysis was determined by standard ^{51}Cr release assay. As shown in Figure 3A, the HIV-1 peptide-specific $CD8^+$ T cells killed A0201 restricted HIV-1 peptide pool-pulsed T2 cells with ~50% specific lysis at an E:T ratio of 30:1. The same T2 cells loaded with HTLV-TAX peptide as a negative control were not lysed. To determine whether HIV-1 peptide-specific A0201 restricted CTL can recognize and lyse HIV-1 -infected cells, we infected the T1 hybridoma line ($A0201^+$, $B5101^+$, $CD4^+$) with HIV-IIIB virus and then determined whether HIV-1 peptide specific A0201 restricted CTL can recognize HIV-IIIB infected T1 cells and kill target cells in a ^{51}Cr release assay. HIV-1 specific CD8 T cells from two different A0201 donors recognized and killed 20–28% of HIV-1 infected T1 cells 2 and 3 days after infection at an E:T ratio of 30:1 while uninfected T1 cells were not lysed (Figures 3B and 3C). We also noted that although some of these peptides (for example, TLNAWYKVI) bind to related alleles, $CD8^+$ T cells stimulated from the A0201* donor fail to specifically lyse peptide-pulsed A0202, A0203 or A0204 targets (Figure 4). Thus, a given CTL response is less degenerate than peptide binding to MHC.

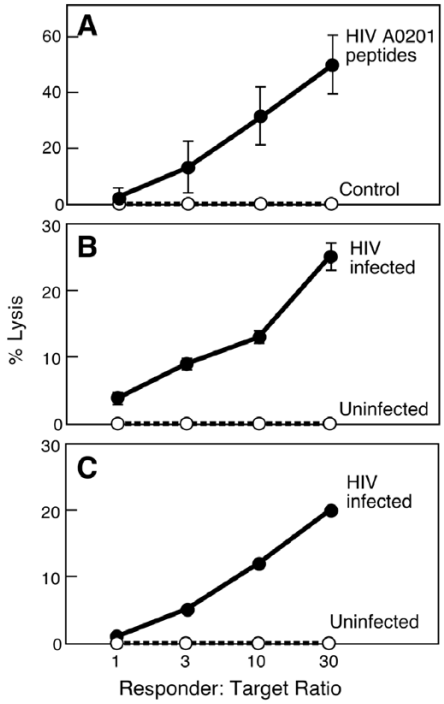

Figure 3
Cytotoxic activity of HIV-1 peptide-specific A0201 restricted T cells from normal donors. CTL were generated against a pool of eight A0201 restricted HIV-1 peptides using PBMC. HIV-1 specific CTL lysed HIV-1 peptide loaded T2 cells (solid symbols) but not the irrelevant peptide loaded T2 cells (open symbols) in ^{51}Cr release assay (Panel A). HIV-1 specific CTL generated from 2 different naïve donors lysed HIV-IIIB- infected T1 cells (solid symbols) but not uninfected T1 cells (open symbols) 2 and 3 days after acute infection, in a ^{51}Cr release assay (Panels B and C, respectively).

As shown in Figure 4, A0201 restricted HIV-1-specific CTL demonstrate alloreactivity and kill A0202, A0203 and A0204 transfected 721–221 targets in the absence of HIV-1 peptide loading. Moreover, in the presence of HIV-1 peptides, alloreactivity against A0202, A0203 and A0204 transfected 721–221 targets decrease as shown by diminished lysis. These results suggest that A0201 restricted HIV-1 peptides bind to A0202, A0203 and A0204, replacing possible endogenous peptides that mediate much of the evident alloreactivity. The analysis of the A0204 transfectant is a clear example of this phenomenon.

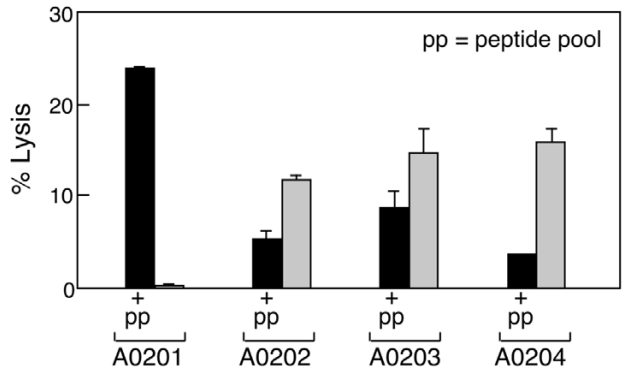

Figure 4
Allele-restricted fine specificity of CTL directed at HIV-1 peptides. HIV-1 peptide-specific T cells were generated from an A0201 donor using predicted A0201-restricted HIV-1 peptides (Table 1 and Fig. 1). HIV-1 peptide-specific T cells killed the A0201 transfected 721–221 cell line in a peptide-specific manner by ^{51}Cr release assay. To test possible cross-reactivity of A0201 restricted HIV-1 peptide-specific CTL to other A02 alleles, we utilized A0202, A0203 and A0204 transfected 721–221 cells as targets. A0201 restricted HIV-1 peptide specific CTLs killed HIV-1 peptide loaded A0201 transfected 721–221 targets at a 10:1 ratio. +pp indicates addition of peptide pool, with solid bar = lysis upon +pp and gray bar = lysis with no peptide pool addition.